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MEDICAL RESEARCH COMMITTEE

Report on the present state of Knowledge concerning Accessory Food Factors (Vitamines)

Compiled by a Committee appointed jointly by the Lister Institute and the Medical Research Committee

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ACCESSORY FOOD FACTORS

CHAPTER I

INTRODUCTION.

THAT substances playing a prominent part in nutrition could have eluded discovery until the arrival of the present decade seemed a short time ago to be quite unlikely: sufficiently unlikely at any rate to induce scepticism towards the subject of this monograph. Nevertheless, while any recognition of the existence and functions of such accessory food constituents as those now termed vitamines is new to the science of nutrition, the facts already available seem to compel belief in their reality and importance. True, there is at present no knowledge concerning their actual chemical nature, but the study of their functions is progressing on real and objective lines, and has become in certain cases even quantitative.

As food constituents the vitamines are characterized by a disproportion between the apparent importance of their functions and the amounts in which they are normally consumed. They are present in quantities far too small to constitute any appreciable contribution to the energy supply of the body. Whether they ultimately prove to be structural components of living tissues of which a supply is essential even though quantitatively unimportant, or whether (as is equally possible) they are found to act rather as catalysts in certain normal processes of metabolism, it seems certain, because they function in such small amounts, that they must be put in a nutritive category different from any which would comprise the better-known foodstuffs such as proteins, fats, and carbohydrates. As already admitted their actual nature is unknown, though much information has been acquired as to their distribution and relations as well as clear evidence that at least three members of the class exist, each with its special function in nutrition.

What can be said precisely at present is that if minute amounts of certain constituents are removed from natural foods, such foods wholly fail to support nutrition, and grave symptoms of actual disease may supervene. The failure and the symptoms may be prevented—or the animal restored to health—by the replacement of what was removed from the food, or by adding an equally small supply from other sources. The constituents that can be so removed are (or rather, contain) vitamines. All the nutritional phenomena observed in connexion with these substances are due to the influence of amounts of actual material so small as to suggest that their functions are of a nature quite distinct from those of proteins or of foods which supply energy. It is not surprising that common experience should have failed to take note of dietetic factors such as these. The vitamines are always present in natural foodstuffs as instinctively consumed by men and animals. There is evidence

to suggest that they are formed only in the tissues of plants, whence they pass into the tissues of herbivorous animals and thus become available for carnivora. Their distribution in the tissues of either plants or animals may be partial and irregular, but broadly speaking it is safe to say that the individual always finds a sufficient supply of vitamines in his food so long as that food is reasonably varied and has received no artificial or accidental separation into parts, and so long as no destructive influence has been applied to it.

In the observation of phenomena the importance of contributory factors is often overlooked until the effects of their removal come to light. In the case of vitamines and their functions, demonstration, so far as it depended upon observation, first came when commercial adventure or other human enterprise had led to the preparation and consumption of foods in which the natural materials had been fractionated for the sake of taste, appearance, or convenience, or treated (over-heated, for example) for preservative purposes. Owing on the one hand to the partial and irregular distribution of vitamines in the natural products, or on the other hand to their instability, it may happen that by these processes they are removed or destroyed.

Safety in this connexion is evidently greater when a variety of foods is consumed. In countries where geographical conditions prevent such variety the danger is more imminent. It is a fact at any rate that the most striking evidence for the evil effects of the artificial treatment of food came from rice-eating districts of the East when they had been invaded by milling machinery from the West. Those who recognize beri-beri as a deficiency disease have good evidence both for the evil effects of fractionating natural foodstuffs such as the cereals, and for the nutritive importance of what we have agreed to call accessory factors.

The evidence from disease would have led sooner to a conception of these food constituents and their functions but for a not unnatural bias in thought. It is difficult to implant the idea of disease as due to deficiency.

Disease is so generally associated with positive agents—the parasite, the toxin, the *materies morbi*—that the thought of the pathologist turns naturally to such positive associations and seems to believe with difficulty in causation prefixed by a *minus* sign. Even in connexion with deficiencies arising within the body there is or was a similar tendency. When the importance of internal secretions was first recognized there seemed to be much hesitation in believing that symptoms might be frankly due to their failure. When each fresh internal secretion was described there was always an effort to show that its function was to ' neutralize ' some, always hypothetical, toxic substance. Symptoms, on this view, were due to the unmasking of a deleterious agent rather than to simple deficiency in a normal and necessary agent. To distinguish between these two possibilities was of course a scientific duty ; but, at any rate in the earlier literature of internal secretions, a bias against the simpler view interfered with the fair interpretation of experimental results.

So in connexion with the newer conception of disease as due to dietetic deficiencies. Even when Eijkman, through his admirable

studies, had clearly established more than twenty years ago that beri-beri arose during the consumption of decorticated and not of whole rice (making it clear, therefore, that something in the cortex was necessary to normal nutrition) he was led to suggest, not the simple view that the cortical substance was of direct use to the body but rather that it was necessary to neutralize the otherwise deleterious effect of a diet over-rich in starch. Save for the mental bias just referred to it is very difficult to see why so roundabout an explanation should have been thought necessary. Its effect in every case has certainly been to delay the recognition of deficiency diseases and indirectly also to delay the realization of the functions of what, for lack of a better term, have been called vitamines.

Fortunately in connexion with deficiency diseases it has proved possible by the choice of suitable animals to supplement clinical and sociological observation by experiments. As usual experimental work has made our knowledge more quantitative. The discovery, for instance, of avian polyneuritis and its relation to beri-beri by Eijkman and the demonstration by Axel Holst that the guinea-pig easily develops the symptoms of scurvy and at the same time responds readily to anti-scorbutic treatment have made possible experimental work upon sound and illuminating lines. In this country the work done in connexion with beri-beri and scurvy at the Lister Institute has shown the value of experiments essentially quantitative in kind. Work on rickets now being carried out at Cambridge has further shown the value of experiment as a supplement to clinical observation when dietetics are concerned. This investigation seems to leave no doubt that a deficiency in the food, almost certainly involving a factor of the vitamine type, plays a fundamental part in the etiology of the disease.

If, however, the evidence for the existence and importance of vitamines had arisen entirely from the study of deficiency diseases our views might have remained too limited, too much confined to the standpoint of pathology and therapeutics. But in the very years during which the etiology of beri-beri was being cleared up and the suggestion established that a quantitative food deficiency may be responsible for striking symptoms, experiments were in progress on quite independent lines to show that something other than a supply of energy and protein is required to maintain so fundamental a physiological phenomenon as growth, and to demonstrate that normal metabolism as a whole is not possible without the influence of food constituents which because of their minute amount could justifiably be spoken of as 'accessory' factors in nutrition.

From such experiments then the conception of the vitamine also arose, and at the present time the evidence from all kinds of feeding experiments, whether carried out from the physiological or pathological standpoint, is being consolidated. In this country valuable contributions to the research are being made at Cambridge, the Lister Institute, the Cancer Hospital, and elsewhere. The work of McCollum and of Osborne and Mendel in the United States has made it clear that at least two substances of the vitamine type are concerned in growth, one of them being probably identical with the antineuritic substance discovered in connexion with beri-beri,

while abundant evidence shows that the antiscorbutic factor is distinct from either of these.

There is no occasion for surprise in the circumstance that students of normal metabolism have, like the pathologist, failed until recently to recognize the existence of such substances in the food. Nearly all the classical experiments have been made upon diets containing natural foods, the content of protein, fat, and carbohydrate being calculated from analysis. Not until these constituents had been thoroughly purified and animals fed upon 'synthetic' dietaries made up of such purified materials could the suggestion arise that the familiar basal constituents of food were not by themselves capable of maintaining life.

A point of interest arises here which is sufficiently cognate to the subject of this monograph to justify some reference to it. The tissues contain, of course, many known organic constituents other than proteins, fats, and carbohydrates. It is clearly a question whether any of them, or the constituent groups of any of them, must be supplied in diet. At one time or another many observations have been made to determine this point. The result of adding certain of these known constituents has sometimes seemed to be positive—in the sense of increasing growth, or of improving metabolism—but the evidence has never been striking or even unequivocal.

Now feeding experiments, such as those which have brought to light the necessity for a vitamine supply, seem to have proved at the same time that all *known* tissue constituents can be synthesized from the amino-acids of protein with the other basal foodstuffs. For, with their vitamine supply secured, animals in great numbers have now been shown to develop normally upon a diet containing only pure protein, fats, carbohydrates, and salts. We must believe, therefore, that substances such as the nucleic acids, the phosphatides, and other complex phosphorus compounds, creatine and the other bases in muscle, as well as many other necessary tissue constituents, must be synthesized from the basal foodstuffs. The vitamines may of course be themselves indispensable tissue elements in the structural sense ; exceptional in that they cannot be synthesized in the animal. If so it would seem that they share that characteristic with certain of the amino-acids of the protein and with those alone. To some, on the other hand, it seems probable that the functions of vitamines are in essence dynamic and catalytic, though the point remains unproven.[1]

The purpose of the present monograph is to present in a collected form the essential facts concerning these accessory diet factors so far as they are at present known. It is hoped that it will be found, in particular, to make readily available information which is already capable of practical application.

The practical importance of the facts will not be understood unless it be recognized that a deficiency in food, which when complete or extreme leads to actual disease, may, when only relative, be responsible for ill health of a vague but still important kind.

[1] So far back as 1911, when Hopkins described his own experiments to a meeting of the Biochemical Society (then the Biochemical Club) the subsequent discussion ranged chiefly round this point. The opinion of those who took part in the discussion was almost entirely in favour of the view that the function of such accessory food factors (as he termed them) must be that of stimulants rather than structural units.

CHAPTER II

ACCESSORY FACTORS AND GROWTH.

The growth of a young animal is believed to be determined by two factors. One of these is what has been termed the growth impulse. This is inborn, and represents the power to grow possessed by individuals of the particular species. It has not yet been possible to resolve and identify the multiplicity of factors which operate to determine the magnitude of the growth impulse, but it may be safely said that the chief variations are those introduced by hereditary influences.

The growth impulse may therefore be termed the internal factor which controls growth and development. The second factor, which may be designated the external factor, must also be regarded as multiple, and is represented by the summation of those external conditions which influence growth. Of the many factors which together constitute this external influence the most important is, without question, the food supply.

Neither of these fundamental factors can be regarded as independent of the other. The growth impulse, predetermined by evolutionary and hereditary influences, represents the power to grow inherent in the fertilized ovum, but this is in itself powerless to promote actual growth unless assisted by the co-operation of the external factor.

On the other hand, the external factor, which for our purpose may be identified with the food supply, cannot stimulate growth beyond those limits which are predetermined by the growth impulse. It will, therefore, be realized what fundamental importance is to be attached to a full understanding of the food requirements of the animal body during the period of growth, since these represent the chief factors which are under voluntary control.

For many years past it has been customary to estimate the nutritive requirements of the animal organism in terms of what have been for long regarded as the four fundamental food units, namely protein, carbohydrate, fat, and inorganic material, and to underestimate, if not entirely neglect, the possible significance of other less clearly defined dietary constituents.

As a result of this narrow conception, we find that only within the last decade has research on animal nutrition advanced beyond the stage when primary importance was attached to the results yielded by elaborate studies of the nitrogen, energy, and mineral exchanges. Whilst acknowledging the great value of the many fundamental principles which have been deduced from such results, much that is misleading and inaccurate has undoubtedly followed from the attempt to allocate to every foodstuff a nutritive value estimated solely in terms of digestible protein, fat, carbohydrate, and salts. It is only necessary to consider the methods by which no inconsiderable proportion of this valuation has been made in order

to appreciate how these errors have arisen; thus, for example, 'protein' is still computed in many cases from determinations of total nitrogen, without any attention being paid to the character of the nitrogen-containing substances actually present in the foodstuffs; whilst the true nature of the substances frequently estimated as 'fat' is almost invariably disregarded.

The sole justification for assuming that proteins, fats, carbohydrates, and salts are all that is necessary for the nutritive requirements of the body can only come from a successful experiment in which an animal has shown a normal development and standard of nutrition throughout its life cycle upon a diet composed of these units in a pure condition.

Early Experiments with Purified Diets.

Many attempts have been made to accomplish this, although it was not an experiment of this nature that led to the first suggestion being advanced that in addition to these recognized dietary units other substances existed, which were equally indispensable for life. This suggestion was made by Lunin as far back as 1881 (1). He was investigating at the time the significance of inorganic salts in the nutrition of the animal, and as a result of some of his experiments found that whereas adult mice could live for several months in good health on a diet of milk, they invariably died within a month if they received a ration composed of what he believed to be the essential ingredients of milk, namely, caseinogen, milk-fat, milk-sugar, and the ash of milk. Commenting upon his experiments, he remarked:

'Mice can live quite well under these conditions when receiving suitable foods (e. g. milk), but as the above experiments demonstrate that they are unable to live on proteins, fats, carbohydrates, salts, and water, it follows that other substances indispensable for nutrition must be present in milk besides caseinogen, fat, lactose, and salts.'

In spite of the fact that this conclusion was given considerable publicity in the widely-read pages of Professor Bunge's *Textbook of Physiological and Pathological Chemistry*, it does not appear to have attracted the attention it merited.

A number of investigators since Lunin's time have attempted to supply the nutritive requirements of animals, usually rats and mice, by means of artificial diets composed of mixtures of proteins, fats, carbohydrates, and inorganic salts, but it was found exceedingly difficult even to keep the animals alive for any appreciable time upon such diets. In practically every case, no matter how carefully the composition of the diets had been planned so as to provide what was considered an adequate and well-balanced supply of the necessary food units, the animals showed a steady decline in weight and health throughout the course of the experiments, and seldom survived for any appreciable length of time.

A valuable review of these earlier experiments is given in the monograph by Osborne and Mendel (2), which should be consulted for fuller details. Many suggestions were advanced to explain the repeated failures. By some it was considered that the monotony of the diet was in itself sufficient to bring about ultimate nutritive

failure; a view that was difficult to reconcile with the fact that animals have been successfully reared and maintained at a normal standard of nutrition for considerable periods upon equally monotonous diets of single foodstuffs such as milk and egg-yolk (3, 4).

Another explanation frequently put forward was that the absence of flavouring agents from the artificial diets induced loss of appetite, with a consequent failure to partake of sufficient food to satisfy the nutritive requirements for maintenance (5). Attempts were therefore made to increase the palatability of the rations by the addition of flavouring substances, but in spite of this, the experimental animals still failed to flourish.

Reference must, however, be made to the experiments of Röhmann, who has done much pioneer work in this field of research. This investigator claimed to have been able to satisfy the nutritive requirements of mice with rations prepared by mixing a number of isolated and ' purified ' food components (6). His animals not only showed increases of body weight upon such diets, but were actually sufficiently well nourished to produce young. A full criticism of his results will be given later, but it may be stated here that there is every reason to believe that he had failed to use sufficient care in ensuring the purity of the components of his food mixtures.

In the year 1911 Osborne and Mendel published in America (2) their classical monograph in which they described experiments on the nutrition of rats over considerable periods upon diets composed of isolated food substances.

They used dietaries containing proteins of a high degree of purity, together with starch, sugar, lard, agar, and inorganic salts. The agar was included in order to form an indigestible intestinal ballast or ' roughage ', whilst the inorganic salts were given either as a mixture of the pure crystalline salts or in the form of a preparation known as ' protein-free milk '. The latter product was prepared by evaporating down milk after removal of the fat and proteins. It therefore contained the salts, lactose, and extractives of milk. With regard to these experiments, they say (2 II. 59) : ' Although these apparently successful experiments indicated that the combinations of isolated food-stuffs employed satisfied the nutritive requirements of the rats, and consequently constituted a complete food for the maintenance of mature animals, a prolongation of the observations has led to a less favourable outcome. A continuation of the experiments over longer periods has shown that in every case, sooner or later, the animal declined ; and unless a change in the diet was now instituted within a comparatively short time, the animals died.'

Mention must now be made of the work of Stepp (7), who found that whereas mice lived satisfactorily for several months upon certain foods, such as wheat bread made with milk, they were unable to live longer than a month when fed upon the same diet after it had been subjected to prolonged extraction with alcohol and ether. That the extraction itself had only lowered the nutritive value of the foodstuffs by removing some essential component was demonstrated by restoring the extract to the extracted food, when it once again became adequate for the nutrition of the mice.

He made a number of experiments in order to ascertain the nature of the essential factor removed by the extraction process. The addition of the ash of the extract, or of certain neutral fats in a pure condition, did not remedy the deficiency, but he found that milk and egg-yolk did. This led him to suggest the existence of an unidentified indispensable dietary unit, which he appears to have regarded as a member of the lipoid class. He was unable to identify this substance with cholesterol, lecithin, kephalin, and cerebrone, since all members of that class of substance failed to restore the nutritive value of the extracted foodstuffs. He remarks in one of his papers: 'It is not impossible that unknown substances indispensable for life go into solution with the lipoids, and that the latter thereby become what may be termed carriers for these substances.'

We must now turn our attention to the classical experiments carried out by Hopkins in this country. As early as 1906 he wrote as follows (8): 'But further, no animal can live upon a mixture of pure protein, fat, and carbohydrate, and even when the necessary inorganic material is carefully supplied, the animal still cannot flourish. The animal body is adjusted to live either upon plant tissues or other animals, and these contain countless substances other than the proteins, carbohydrates, and fats. Physiological evolution, I believe, has made some of these well nigh as essential as are the basal constituents of diet; lecithin, for instance, has been repeatedly shown to have a marked influence upon nutrition, and this just happens to be something already familiar, and a substance that happens to have been tried. The field is almost unexplored, only it is certain that there are many minor factors in all diets, of which the body takes account. In diseases, such as rickets, and particularly in scurvy, we have had for long years knowledge of a dietetic factor, but though we know how to benefit these conditions empirically, the real errors in the diet are to this day quite obscure. They are, however, certainly of the kind which comprises these minimal qualitative factors that I am considering. Scurvy and rickets are conditions so severe that they force themselves upon our attention, but many other nutritive errors affect the health of individuals to a degree most important to themselves, and some of them depend upon unsuspected dietetic factors.'

The results of his experiments extending over several years were published in 1912 (9). He fed young rats upon an artificial food mixture containing caseinogen, starch, cane sugar, lard, and inorganic salts. When the animals were fed upon the diet composed of these constituents in the crude condition, they were able to live and to show a certain amount of growth. When, however, the components had been carefully purified, growth invariably ceased after a comparatively short period, and the rats declined and died. A most important feature of his experiments was that he showed by estimations of the energy consumptions of the animals that this failure was not due to an insufficient food intake. It was found that they ceased to grow at a time when they were consuming food in more than sufficient quantity to maintain normal growth.

Another series of animals received in addition to the basal ration

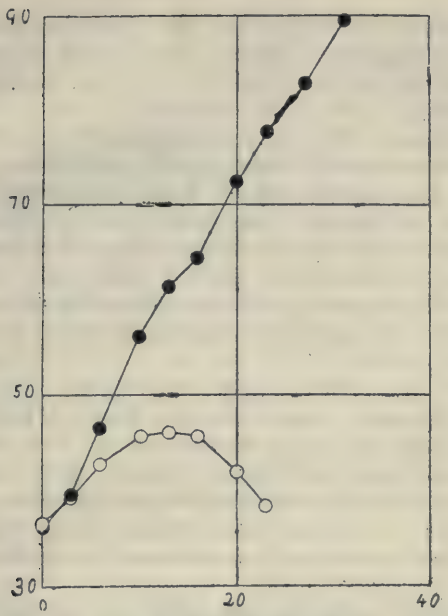

FIG. 1. Lower curve, six rats on artificial diet alone. Upper curve, six similar animals receiving in addition 2 c.c. of milk each per diem. Abscissae, time in days; ordinates, average weight in grm. (Reproduced by permission from the *Journal of Physiology*, 1912 **44** 432.)

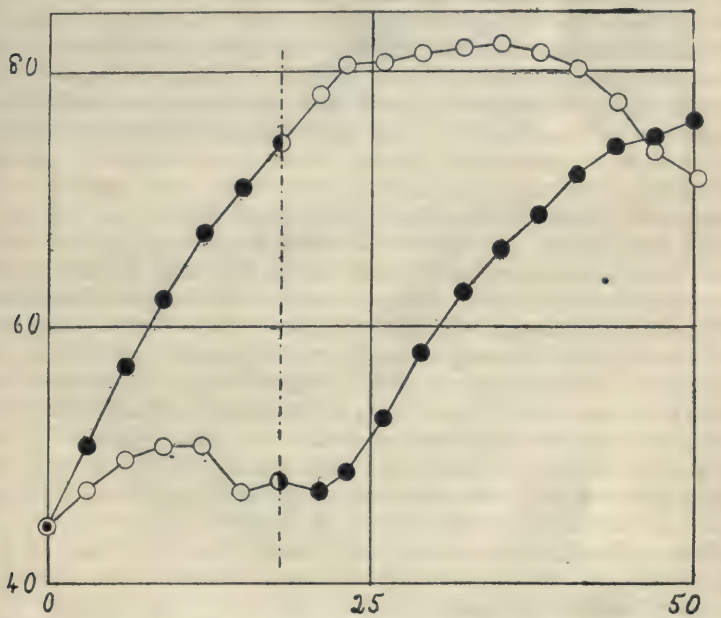

FIG. 2. Lower curve (up to eighteenth day), eight male rats upon pure dietary; upper curve, eight similar rats taking 3 c.c. of milk each a day. On the eighteenth day, marked by vertical dotted line, the milk was transferred from one set to the other. Average weight in grms., vertical; time, horizontal. (Reproduced by permission from the *Journal of Physiology*, 1912 **44** 433.)

of purified foodstuffs a very small daily allowance of milk. In all cases the milk addendum, although its total solids amounted to only 4 per cent. or less of the whole food eaten, induced normal and continual growth.

The extraordinary effect of this apparently insignificant addition to the diet upon the growth of the experimental rats is well illustrated by the above two figures, which have been reproduced from the original paper. A similar growth-stimulating action was exerted by the addition of protein-free and salt-free extracts of milk-solids, or of yeast, to the basal diet. As the milk ration was administered separately and in advance of the administration of the main dietary, it could not have affected the palatability of the food or diminished its monotony.

The importance of these early experiments is so great that an extract from Hopkins' discussion of his results is reproduced : ' It is possible that what is absent from artificial diets and supplied by such addenda as milk and tissue extracts is of the nature of an organic complex (or of complexes) which the animal body cannot synthesize. But the amount which seems sufficient to secure growth is so small that a catalytic or stimulative function seems more likely.

' It is probable that our conception of stimulating substances may have to be extended. The original vague conception of such subjects being condiments, chiefly affecting taste, gained in definiteness by the work of the Pavlov School. But the place of specific diet constituents which stimulate the gastric secretory mechanism can be taken by the products of digestion itself, and in this connexion the stimulant in the diet is by no means indispensable. Most observers agree that the addition to normal dietaries of meat extracts capable of stimulating the gastric flow does not increase the actual absorption of food, though this point could be properly tested by adding them to an artificial dietary known to be free from analogous substances. As was emphasized above, the milk did not affect absorption in my experiments. But such undoubted stimulating effects due to diet constituents as those discovered by Pavlov may quite possibly be paralleled elsewhere in the body on more specific and indispensable lines. Stimulations of the internal secretions of the thyroid and pituitary glands which are believed on very suggestive evidence to play an important part in growth processes, can be legitimately thought of. On the other hand, the influence upon growing tissues may be direct. *If the attachment of such indispensable functions to specific accessory constituents of diets is foreign to current views upon nutrition, so also is the experimental fact that young animals may fail to grow when they are absorbing daily a sufficiency of formative material and energy for the purpose of growth*'.

These experiments undoubtedly mark the beginning of a full appreciation of the importance of what Hopkins has termed the *accessory factors of the diet.*

Additional evidence of the existence of such factors rapidly accumulated ; Osborne and Mendel (10) confirmed their previous finding that when they replaced ' protein-free milk ' as a source of carbohydrates and salts in their artificial food mixture by lactose

and a mixture of pure inorganic salts approximating to the composition of the ash of milk, the animals were unable to thrive. Summing up they say, ' whether the deficiency of the purely artificial diet is to be attributed to improper proportions of its constituents, to improper combinations of these constituents, or to the lack of some essential element, is at present difficult to define.'

That they did not regard their failure as being entirely due to the absence of accessory factors may be assumed from their statement that they had achieved a considerable degree of success in feeding in the absence of the hypothetical organic hormones (10). Hopkins and Neville (11) challenged this statement and attempted to repeat the experiments of the American workers. Using very carefully purified ingredients they were unable to obtain any appreciable growth on such diets, and the animals, in spite of a satisfactory consumption of food, were all dead before the expiration of forty days. In complete confirmation of the earlier experiments of Hopkins, it was found that when a small ration of milk, 2 c.c. per day was given, in addition to the basal diet, this failure was not shown.

Differentiation of Two Accessory Factors.

In 1913 McCollum and Davis in America confirmed the fact that rats were unable to grow upon an artificial ration consisting of caseinogen, lactose, lard, and inorganic salts (12). They also stated their belief that cessation of growth upon such diets was due to the animal running out of some organic complex which was indispensable for further growth, but since their rats lived in fairly good health upon this mixture after growth had ceased, they concluded that maintenance could be satisfactory in the absence of that substance. They found that the ether-soluble fraction of butter and eggs supplied the missing factor, but that lard and olive oil were unable to do so.

In concluding their paper, they remark : ' Our observation that ether extracts from certain sources improve the condition of animals on such rations strongly supports the belief that there are certain accessory articles in certain foodstuffs which are essential for normal growth for extended periods.'

Very shortly after the appearance of this paper, Osborne and Mendel reported a continuation of their experiments (13). The greater efficiency of natural milk as a food, as compared with artificial mixtures containing what were supposed to be the only constituents of milk, impressed them with the belief that there existed some unidentified substance in the natural product which was not only necessary for growth but also for prolonged maintenance. They found that young rats which fed upon a diet containing protein, starch, lard, and protein-free milk grew at a satisfactory rate for a short time, but that sooner or later growth invariably ceased and that the animals declined. This decline could, however, be arrested and a normal rate of growth resumed when butter was substituted for the lard in the diet.

Later these investigators found that the active substance was concentrated in the butter-fat fraction of the butter (14). Since

the authors claimed that their butter fat was free from nitrogen and phosphorus and devoid of any ash-yielding or water-soluble components, it appeared probable that the growth factor was not a substance of the type to which the anti-beri-beri factor, then being carefully studied by Funk, apparently belonged. The latter worker, however, challenged their claim as to the purity of butter fat, and showed that appreciable traces of nitrogen-containing substances could be extracted from that product prepared by the method described by Osborne and Mendel (15).

Numerous naturally occurring fats and oils were examined for the presence of the substance which apparently played so important a part in the nutrition of the animal body, and it was found in association with several animal fats, such as cod-liver oil and beef fat, but was absent from a number of oils of vegetable origin (16, 17). The distribution of this substance will, however, be more fully considered in a later section.

For some time it was generally believed that the substance found in association with certain fats was the only accessory substance necessary to supplement the nutritive deficiency of a diet of purified proteins, fats, carbohydrates, and salts for growth. Several workers had, however, failed to secure the satisfactory nutrition of animals even when butter fat was added to the basal ration of purified food-stuffs. Thus Funk and Macallum in 1915 reported that young rats fed upon such a diet not only failed to grow but rapidly declined and died after many of them had shown symptoms which the authors believed to be analogous to those of avian beri-beri (18). On the basis of Funk's vitamine theory of beri-beri (see later) they added a small ration of yeast to the diets and found that this addition was immediately followed by growth.

They failed, however, to appreciate the true significance of their own results, for they apparently disregarded the influence of any substance present in the butter fat, and expressed their opinion that ' the growth-promoting factor is beyond question contained in the yeast '.

The proof of the existence of a second essential accessory dietary factor of a type distinct from that found in association with fats was first given by McCollum and Davis (19). As the result of a careful investigation of the dietary deficiencies of rice they were forced to accept the conclusion ' that there are necessary for normal nutrition during growth two classes of unknown accessory substances, one soluble in fats and accompanying them in the process of isolation from certain food-stuffs, and the other soluble in water but apparently not in fats '. They termed these two substances ' fat-soluble A ' and ' water-soluble B ' respectively ; preferring these provisional appellations to the much criticized word ' vitamine ', which had been introduced and used extensively by Funk. Their terminology has been widely adopted as being both convenient and non-committal.

Amongst other interesting points they showed that the so-called water-soluble factor is present in milk, and that it is only removed from milk sugar by thorough crystallization, a fact confirmed by Drummond (20).

This observation supplied the solution to the apparent discordancy of many of the experimental results which had been obtained by various investigators, of whom many had employed in one form or another insufficiently purified lactose as a component of their dietaries, thereby unwittingly providing a more or less adequate supply of the water-soluble accessory factor. Thus, Funk and Macallum, who had taken great pains to ensure the purity of the components of their food mixture, and who excluded lactose on the grounds that it was known to contain traces of nitrogenous products derived from milk, were unable to obtain growth in the presence of butter fat until they supplied yeast, now known to be one of the richest sources of the water-soluble factor.

Practically all investigators in this field of research have now admitted their belief in the indispensability of the two accessory factors, 'fat-soluble A' and 'water-soluble B' for growth and nutrition of the animal organism, but a brief reference must be made to certain opinions which have been advanced in opposition to this view.

Foremost amongst the very small minority who have not yet acknowledged the indispensability of these constituents is F. Röhmann of Breslau. As the majority of his views and criticisms are contained in a book published in Germany in 1916 (21), and as no copy of this publication has as yet been available for reference in this country, we are unfortunately confined mainly to an examination of the criticisms it has roused in America (22). Apparently Röhmann asserts his belief that 'accessory foodstuffs are not necessary for the continued maintenance of fully grown animals', and that if the long familiar nutriments are suitable in quality and quantity, nothing further is essential to the ration. He remarks: 'The assumption that some unknown substances are indispensable for growth is a convenient device for explaining experiments that result in failure—a device that becomes superfluous as soon as the experiment succeeds.' But we must first examine his own experiments which he claims have been successful in achieving this result and which have led him to express these views.

Röhmann has for many years past carried out a large number of feeding experiments with artificial mixtures of foodstuffs, and has thereby obtained valuable results upon such questions as the relative nutritive values of individual proteins. It is impossible to say, however, that he has exercised sufficient care in controlling the purity of the ingredients of his food mixture. Many of his dietaries contained protein in a very impure form, such as crude dried egg albumin and 'Kalzose' the latter a commercial preparation of calcium-casein, admittedly containing milk sugar, and hence without doubt other products present in milk. The composition of the margarine, which he frequently employed as a source of fat, is never stated. It may, if derived from animal fat, contain large amounts of fat-soluble A (24). That he has failed to appreciate the difficulty of removing traces of the accessory substances from the ingredients of his diets may be judged from the fact that, in order to meet the possible objection that his diets contained these substances in some cases, he only extracted the components with cold alcohol. More

astounding is his admission that a successful nutrition with certain diets was frequently induced by the addition of alcoholic extracts of yeast or by small quantities of a product termed by him ' Filtrateiweiss '. Of this latter substance, Osborne and Mendel remark : ' according to his (Röhmann's) account of the method of preparation, this product may have contained some or all of the other constituents of milk among which are those proved to be especially efficient in promoting growth.'

It is scarcely necessary to expend further time in considering work so full of inconsistences, and we may conclude by quoting the trenchant criticism of the American workers (22). ' The thesis that a successful, i. e. positive, experiment in nutrition is far more significant than a negative one is doubtless valid. On the other hand, in dealing with substances which, like the alleged vitamines, are potent in surprisingly small amounts, the burden of the proof with respect to the complete absence of effective substances so widely distributed among the natural foodstuffs falls on those who deny the need of them.'

Are Fat-soluble A and Water-soluble B the only Accessory Substances Necessary for Growth and Nutrition?

A later section of this book is devoted to a consideration of a third accessory substance, which may be sharply differentiated from both the factors ' A ' and ' B ' by its properties, namely the antiscorbutic substance. It will be seen there that this factor is undoubtedly indispensable for the normal nutrition of certain species of animals.

The question, therefore, arises as to whether we must regard this substance as a third accessory factor indispensable as a component of a normal dietary for growth. It is difficult to give an answer to this question in the light of our present knowledge of the subject, because whilst we as yet understand very little of the requirements of the various species for this substance, it is undoubtedly true that wide variations occur.

Practically all the researches which have led up to the discovery of fat-soluble A and water-soluble B have been carried out on young mice and rats. Neither of these animals appears to show any susceptibility to scurvy, if we may judge from the fact that they have been nourished with apparent success over long periods upon diets which can have contained but the merest traces, if any, of the anti-scorbutic substances. It is, however, quite conceivable that these two species represent types whose low susceptibility to the disease calls for a very small requirement of the preventive substance. Failure to supply an adequate amount of the factor in such cases might only bring about a comparatively slight lowering of the nutritive standard of the animal, and experimental evidence is accumulating that this is actually what occurs in the case of the rat (23, 23 a). Strictly speaking, we must recognize at least three distinct accessory factors indispensable for normal growth and nutrition, but as the anti-scorbutic substance is fully treated elsewhere, the other two substances, fat-soluble A and water-soluble B, will alone be considered here.

Illustrations of the Influence of Fat-soluble A and Water-soluble B on the Growth and Nutrition of Rats.

In Fig. 3 are given a number of curves illustrating the changes in body weight of rats which were fed from an early age upon an artificial ration of purified protein, carbohydrate, fat and inorganic salts, supplemented with adequate amounts of the factors 'A' and 'B'.[1]

The actual dietary employed was constituted as follows:

Purified caseinogen	20 parts
„ starch	55 „
Mixture of crystalline inorganic salts	5 „
Butter fat (as source both of fat and the factor 'A') .	15 „
Yeast extract (as a source of factor 'B') . . .	5 „

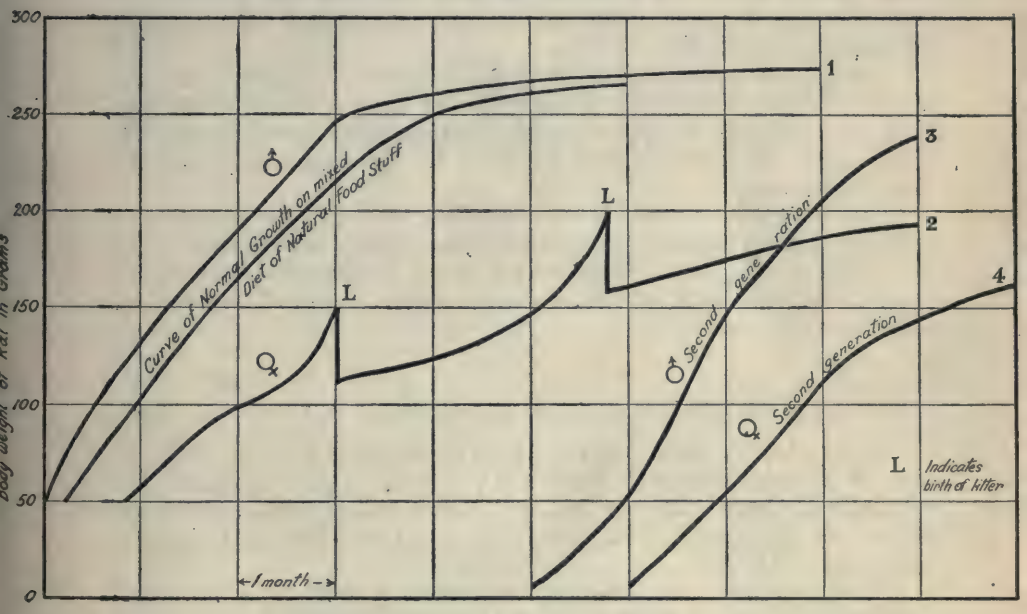

Fig. 3. Showing normal growth of rats over 4–5 months with production, and satisfactory growth, of young on above dietary.

Curve 1 represents the growth of a male rat, and Curve 2 the growth of a female rat. At the points marked on the second curve litters of young were born. Average curves for the growth of the second generation reared on this diet are given in Curve 3 (males) and Curve 4 (females). In one experiment four generations were reared upon this dietary as a sole source of food, and in every case the animals were well up to, and in some cases were considerably superior to, the normal standard.

[1] The curves illustrating this section are taken from records obtained during experiments carried out by Drummond. They confirm those previously obtained by McCollum and his co-workers.

The composition of the salt mixture employed is one recommended by McCollum and Davis and is as follows (25) :

NaCl	5·19 parts
$MgSO_4$	7·98 ,,
NaH_2PO_4	10·41 ,,
K_2HPO_4	28·62 ,,
$CaH_4(PO_4)_2$	16·20 ,,
Ca lactate	39·00 ,,
Ferric citrate	3·54 ,,
Iodine	trace.

In addition to the above the animals received occasional doses of orange juice as an antiscorbutic.

That the basal ration of protein, starch, and salts was free from accessory substances is shown by complete failure of young animals to grow when fed upon it, illustrated by the curves given in Fig. 10 (p. 19).

Throughout this experiment the rats were apparently in excellent health and showed a normal appearance. A rat from this group was photographed after having received the diet for over twelve months (Fig. 4) ; one of the second generation raised on this ration is also shown (Fig. 5).

From the close approximation of the weight curves of these animals to the standard curve for similar rats fed upon a mixed dietary of natural foodstuffs it appears probable that their nutritive requirements had been completely supplied by the components of the artificial dietary.

This is supported both by the fact that the experimental animals showed normal breeding propensities, many highly satisfactory litters of young having been produced and reared by females receiving the artificial ration, and by the animals showing a normal standard of resistance to disease.

Influence of the Absence of Fat-soluble A from the Diet.

It has already been remarked that certain fats are very deficient in the fat-soluble factor. Such a fat is lard. Fig. 6 represents the changes in body weight of rats fed upon a ration similar to that of which the composition has been given, but in which lard replaced the butter fat.

It will be seen from these curves that for a short time the young animals are able to grow when the fat-soluble factor is deficient in their diet. The explanation of this is probably supplied by the assumption that the animal organism normally contains reserve supplies of the factor ' A ' in its own body. If this hypothesis is correct it is reasonable to assume that these reserves are mobilized for use when a deficiency occurs in the diet, but as soon as they are exhausted growth is immediately inhibited. During the period of temporary growth, throughout which it has been assumed that the reserve supplies are being utilized, the animals show a normal appearance and there does not appear to be any decline in their health, but when the reserves are exhausted and the deficiency becomes felt, not only do

FIG. 4. Male rat reared from the age of five weeks upon the complete artificial ration described on p. 15.

The animal is in perfect condition and has shown normal development for over twelve months on the diet.

FIG. 5. Young rat, one of a litter produced by a female rat which had received the complete artificial ration since she was five weeks old. The father of this rat is shown in Fig. 4. The young rat is in perfect health and is well above the average weight for its age (eight weeks).

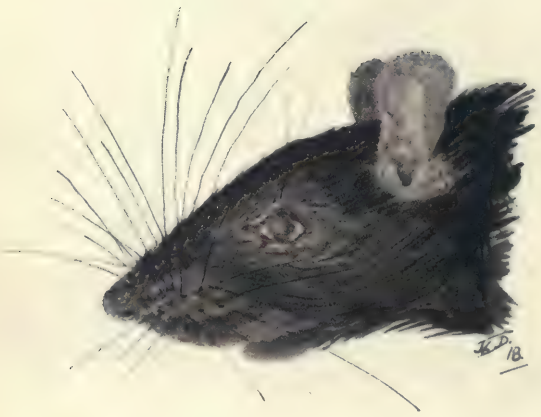

FIGURE 7.

they cease to grow but they become highly susceptible to bacterial infection. In the case of rats this lowered resistance first becomes apparent in many cases by the appearance of a characteristic infection of the external eye, which has been provisionally classified as a xerophthalmia. It usually begins with a swelling of the lids of one or both eyes, which is followed by an inflamed and catarrhal condition of the conjunctivae (Fig. 7). This rapidly becomes worse and the discharge which is at first haemorrhagic, frequently becomes purulent. If untreated, the cornea may be involved and total blindness result. This condition is regarded by some (e. g. McCollum) as an almost

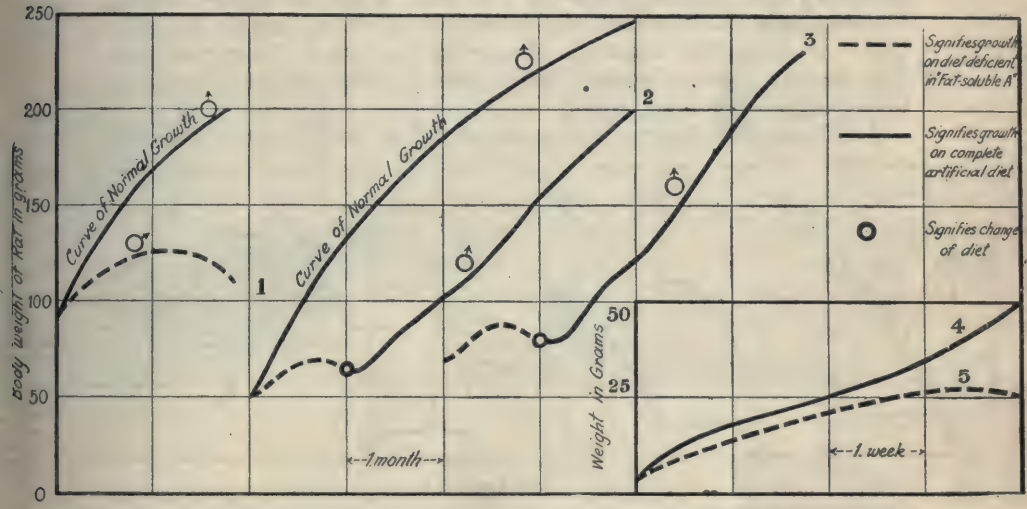

Fig. 6. Showing preliminary growth and eventual decline on diet deficient in Fat-soluble A. Also recovery on addition of a source of Fat-soluble A.

These curves illustrate how the absence of the fat-soluble accessory substance ultimately inhibits the growth processes of young animals. The broken curves represent the changes in body weight of rats fed upon a diet freed as far as possible from all traces of that accessory factor. The small amount of growth, which usually occurs at first, probably indicates the utilization of reserve stores of the indispensable unit, but failure ultimately sets in. At the points marked on the curves by (o) the deficiency of the fat-soluble was made good by the addition of butter fat. This change was at once followed by a resumption of growth. In the small figure are given the weight curves of young rats born from females receiving artificial diets of this nature. Curve 4 indicates the normal growth of young rats nursed by mothers receiving an adequate artificial ration similar to that used in the experiments illustrated in Figure 3. Curve 5 shows the development of young nursed by females receiving a dietary deficient in the fat-soluble factor. These two curves represent the average of a considerable number. The young nursed by the females on the inadequate ration were for some time not much below the normal standard as regards body weight, although they were at all times markedly undernourished.

specific result of a deficiency of the fat-soluble factor. This view is certainly supported by the observation that if animals showing these symptoms be given a diet containing an adequate amount of the fat-soluble substance, the conjunctivitis usually clears up within a few days and the animal is very soon able to resume a normal rate of growth. An example of this recovery in growth is illustrated in Fig. 6. Fully-grown animals are apparently able to live for periods of many months in the absence of the dietary essential. Sooner or

later, however, the deleterious influence of the deficiency is made apparent by the appearance of the external eye disease, or by a greatly lowered general state of health, resulting in an abnormally high death rate from acute infections particularly those affecting the lungs.

Influence of the Absence of Water-soluble B from the Diet.

Contrary to the case of the fat-soluble factor, the animal does not appear to possess any appreciable reserve store of the water-soluble accessory upon which to draw in the event of an emergency arising. Consequently the ill-effects of a deficiency are more quickly apparent (26). An immediate cessation of growth is shown by young animals, followed by a short period during which body weight is more or less satisfactorily maintained.

Adult animals show a very gradual fall in body weight following

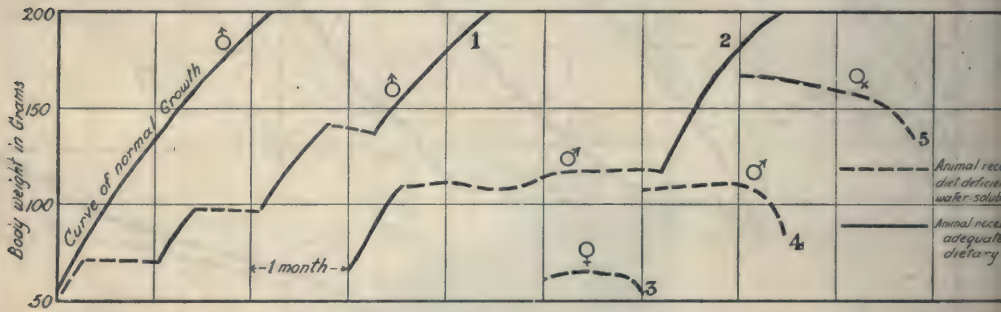

Fig. 8. Showing failure to grow on diet deficient in water-soluble ' B ', and recovery on adding that factor.

These curves illustrate how a deficiency of the water-soluble growth factor immediately causes a cessation of growth, and show the equally prompt resumption when ar extract containing the active substance is added to the dietary of the experimental animal.

Curves 3, 4, and 5 demonstrate that the more mature the animal, the longer can it survive against the deleterious effects of a deficiency of water-soluble A.

the restriction, but sooner or later both young and old subjects suffer a rapid decline which invariably terminates fatally.

Body weight curves illustrating these observations are given in Fig. 8.

A number of the rats dying as a result of this deficiency have been observed to show symptoms of muscular inco-ordination due to involvement of the nervous system (see p. 35). Immediate recovery is effected by administering the missing factor, provided the condition of the animal is not extreme (Fig. 8).

Fig. 9 illustrates the condition of a rat deprived of ' water-soluble B '.

Influence of the Absence of both Fat-soluble A and Water-soluble B from the Diet.

When both these important constituents are absent from a diet it is usual to find that the deficiency of the second named is that which first exerts its deleterious action upon the growth and health

FIG. 9. This photograph represents the condition shown by a young rat which is suffering from a deficiency of water-soluble B. There is no co-ordination of the movements of the hind quarters and the animal is unable to use the hind legs, which may be seen stretched out in a helpless manner. This animal showed a rapid recovery from this condition and was able to walk about with ease twenty-four hours after a dose of yeast extract had been given by the mouth.

[*To face p.* 18.

of the animal. This may be satisfactorily explained on the assumption that the body normally contains an available store of fat-soluble A. A deficiency of the latter, therefore, does not arise until the available reserves have been utilized, by which time the inadequate supply of the factor 'B' has induced a cessation of growth and a serious disturbance of the general health.

Young rats fed upon a diet inadequate as regards both these factors immediately cease to grow (see Fig. 10), and usually die showing symptoms associated with an absence of the water-soluble component, seldom surviving sufficiently long to exhibit the characteristic symptoms associated with a deficiency of the fat-soluble substance.

Physiological Significance of Fat-soluble A and Water-soluble B.

1. *Fat-soluble* A. This factor in all probability is synthesized by the plant, and is found to be present in green leaves and in the

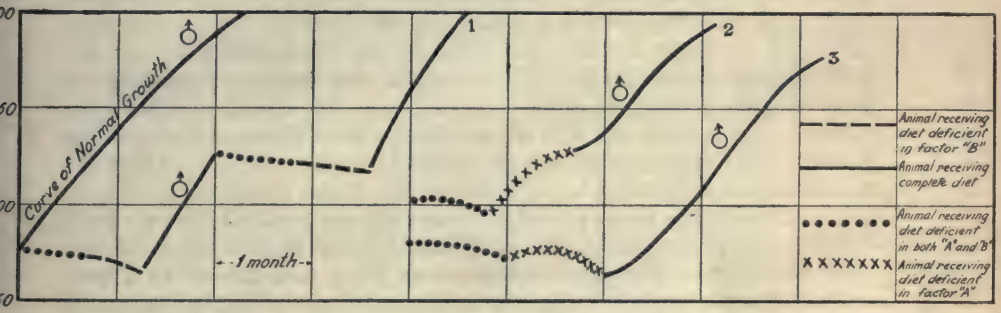

Fig. 10. Growth curves of rats fed on diet deficient in both fat-soluble A and water-soluble B.

The curves represented by (. . . .) show the inability of young animals to grow when both factors, A and B, are absent from the diet. When A alone is added, as for instance by the inclusion of butter fat in the ration (curves marked - - -), matters are not improved, but an immediate resumption of growth follows the restoration of both the missing units (curves marked ——).

When water-soluble B alone is added there is occasionally slight growth (curves marked x x x x), probably as a result of the utilization of reserve stores of the factor A, but it seldom lasts for long and is eventually followed by failure.

embryos of many seeds. In the latter locality it is perhaps present in the form of a loose type of combination with some cell-constituent other than fat. This conclusion has been arrived at from the observation that the simple processes used to extract fats, such as pressure or extractions with solvents, do not remove the fat-soluble factor from seeds, and this accounts for the absence of the factor from the vegetable oils prepared for consumption by these methods (17). The combination, whatever its actual nature may be, is very readily broken up. Treatment of the finely divided seed embryos with alcohol will effect the rupture, and subsequent ether extraction will remove not only the fats but the fat-soluble accessory substance.

We as yet know nothing definite of the part it plays in the nutrition of the body. There is every reason to believe that considerable amounts may be stored, perhaps in association with the reserve fat

supplies, but this has not been experimentally proved. The fat derived from many tissues also contains the factor, so that it would appear to be closely associated with fat during transport of the latter in the body.

The suggestion was at one time advanced that the fat-soluble accessory substance was a characteristic accompaniment of fats, such as milk-fat and egg-fat, elaborated by active organs for the express purpose of providing for the nutrition of the young, and that it was absent from purely depot fats, such as lard. This theory, however attractive, became untenable after it had been shown that the depot fat of oxen contained the factor in considerable amounts. No entirely satisfactory explanation of the deficiency of lard has yet been advanced.

Whether the factor is intimately concerned in the metabolism of fats, or whether it plays some more direct part in the nutrition of the cell is as yet uncertain, but there is a certain amount of indirect evidence in favour of the latter view. There is apparently little appreciable loss of body fat in animals showing the characteristic symptoms associated with a deficiency of the fat-soluble substance, and such cases do not appear to show any obvious signs of a disturbed fat-metabolism (27). Storage of the factor appears to be particularly marked in adult animals, and the requirements, after maturity is reached, are of a smaller order than those of the growing young. The fully-grown rat is able to live for periods of several months upon what may be regarded as an almost complete deficiency of the fat-soluble factor, without showing appreciable loss of body weight or serious symptoms of ill-health, but at the same time it may be inferred that such animals are being maintained at a subnormal level of nutrition by their decreased powers of propagation and increased susceptibility to infection.

The storage of the fat-soluble factor is of considerable importance to the pregnant and nursing mother, for she is thereby enabled to provide her young with a high concentration of this factor without immediate dependence upon an external supply. The importance of this cannot be over-estimated, and it has been suggested that the ill effects which follow a deficiency of the fat-soluble substance are much more serious and far-reaching when they occur in early life, particularly during the period when the young animal is dependent upon its mother (28).

2. *Water-soluble* B. The plant kingdom also provides the primary source of the water-soluble substance. The power to synthesize this substance is possessed generally by plants, even by some which appear low down in the natural classification. Thus, for example, yeast constitutes one of the richest known sources of the substance.

It apparently exists free in the plant cell, although it has been stated that extracts of certain natural foodstuffs are more active after treatment with dilute acids : confirmation of this observation would tend to indicate the existence of the factor in some combination from which the acid liberates it.

Of its action following absorption from the digestive tract, little, if anything, is known. The type of nutritive failure and the production of symptoms of nervous inco-ordination, produced by

a deficiency of this factor, have already been described. A study of the onset of these ill-effects does not indicate that the normal animal possesses any large reserve of the water-soluble factor in an available form. The requirements of the body for the factor are much greater during growth than during maturity. As in the case of the fat-soluble substance, the quality of the milk of the nursing mother will suffer if her diet becomes deficient in this dietary essential. The adverse effects of this deficiency are, however, much more rapidly apparent in this case, for she has little, if any, reserve supply available in her own tissues. This point will receive further attention in a later section (p. 70).

Distribution of Fat-soluble A and Water-soluble B in Natural Foodstuffs.

1. *Fat-soluble* A. The presence of the fat-soluble factor was first detected in butter and egg-yolk, and, as far as is at present known, these foodstuffs represent the richest sources of that substance. It has also been found present in many oils and fats derived from the animal kingdom, as for example, cod-liver oil, shark-liver oil, beef fat, the fats of kidneys, heart muscle and liver tissues, herring oil, cod oil, salmon oil, and whale oil.

On the other hand, the following oils and fats, all with one exception, derived from plant sources, contain only small amounts or are deficient in the accessory factor; sunflower-seed oil, corn oil, olive oil, cotton-seed oil, almond oil, arachis or peanut oil, linseed oil, coco-nut oil, and lard. Since there is good reason to believe that the animal organism does not possess the power to synthesize either of the factors A or B, it must derive its supplies of these substances from outside sources. The primary sources of fat-soluble A are found in the green leaves of plants, and the embryos of certain seeds. The former appear to be the richer source, but very few quantitative data upon the distribution of the substance have yet been obtained. It is, therefore, difficult to attach a definite value to any individual foodstuff as a source of fat-soluble A. This is particularly true in the case of the foodstuffs of animal origin, as their value as sources of that factor is in all probability directly determined by the nature of the diet which the animal has previously received (28). Thus, the milk yielded by a cow which has for some time past been receiving a diet deficient in fat-soluble A, will, without doubt, sooner or later show the same deficiency.

The following tabulation of the chief foodstuffs has been made with the object of illustrating the distribution of the fat-soluble A factor. In the absence of quantitative data it has been impossible to do more than to indicate the relative values of the foodstuffs as sources of the accessory factor by the rough method of positive and negative signs. An attempt to give some idea of relative values has been made by the employment of more than one such sign in certain cases. A zero indicates the absence of the factor. (See also the combined table in which the anti-neuritic (water-soluble) and anti-scorbutic factors are included, p. 50.)

Table I

Class of foodstuff.	*Individual foodstuff.*	*Fat-soluble* A.
Fats	Butter	+ + +
	Cream	+ +
	Margarine prepared from animal fats other than lard. (Value is in proportion to quality and percentage of animal fat.)	+ +
	Margarine prepared from vegetable fats or lard	0
	Nut butter	Variable.
	Vegetable oils such as are commonly used for salad oils or frying oils (e. g. olive and cotton-seed oils)	0
	Hardened fats, such as are sold as lard substitutes	0
	Cocoa butter	0
	Coco-nut oil	0
	Linseed oil	0
	Lard	0
	Mutton and beef fat	+ +
	Cod-liver oil and other fish liver oils	+ + +
	Herring oil, salmon, or cod oil	+ +
Meat and fish	Lean meat (beef or mutton)	Inconclusive result.
	Liver	+ +
	Kidneys	+ +
	Heart	+ +
	Brain	+
	Blood	Inconclusive result.
	Sweetbreads	+
	'Lean' fish, such as cod, haddock	0
	'Fat' fish, as herring, salmon	+ +
	Fish roe	+
Cereals	Wheat embryo	+ +
	„ endosperm	0
	„ bran	Inconclusive result.
	Maize embryo	+
	„ endosperm	0
	Rice embryo and bran (i.e. so-called rice polishings)	+
	Rice (polished)	0
	Wholemeal bread	+
	White bread	0
	Custard powders and egg substitutes	0
	Millet	+ +
	Linseed	+ +
	Linseed cake (after expulsion of oil)	+
Legumes	Peas	+
	„ (dried)	Inconclusive result.
	Kidney beans	+
	Soy-beans	+
Other vegetables and fruit	Cabbage (fresh)	+ +
	„ (dried)	+
	Lettuce	+ +
	Spinach	+ +
	Carrots (fresh)	+
	„ (dried)	+
	Onions	+
	Tomatoes	(?)
	Potatoes	+
	Oranges	0
	Bananas	+
	Apples (green)	0
	Nuts (Walnuts)	+
Miscellaneous	Cheese (prepared from whole milk)	+ +
	„ (prepared from skim milk)	0

Class of foodstuff.	*Individual foodstuff.*	*Fat-soluble* A.
Miscellaneous	Eggs (yolk)	+ + +
	„ (white)	0
	„ (dried)	+ +
	Yeast (dried)	Very slight, if any.
	„ (extract) (commercial)	0
	Meat extract (commercial)	0
	Malt extract „	0

2. *Water-soluble* B. In view of the probable identity of this with the anti-neuritic factor, the distribution of the two is treated on pp. 27 and 35.

Properties of the Accessory Substances, Fat-soluble A and Water-soluble B.

1. *Fat-soluble* A.

(*a*) When associated with fats it is not extracted by water, but is soluble in solvents which dissolve fats, e. g. ether, ligroin, and alcohol. It cannot, however, be extracted by fat solvents from green leaves.

(*b*) It was at one time generally believed that the fat-soluble accessory factor was comparatively stable to heat. Thus, Osborne and Mendel (30) recorded that butter fat treated with steam for two and one-half hours did not appear to have lost its value as a source of the factor. Indirect evidence which supported that belief was given by the observation of Drummond (31), who found that certain fish oils, such as crude whale oil, which are frequently prepared by processes involving the use of high pressure steam for several hours, contained considerable amounts of the factor.

More recent work has, however, cast some doubt on the stability of this accessory toward heat (32). The results of this investigation, carried out in America by Steenbock, Boutwell, and Kent, indicate that the fat-soluble accessory substance is gradually destroyed at 100° C., and that four hours' exposure to that temperature is sufficient to render butter fat of little greater nutritive value from the standpoint of vitamine supply than an equivalent quantity of lard.

This result has now been confirmed by experiments of a similar character carried out in this country (33).

(*c*) It is completely destroyed during the 'hardening' of oil by the action of hydrogen, a process now widely employed for the preparation of edible fats (33).

(*d*) It has been stated to be stable to alkali under the conditions employed for hydrolysis of fats at room temperature in a non-aqueous solution (34).

2. *Water-soluble* B. The properties of this factor are, as has already been remarked, identical with those described for the anti-beri-beri substance (29), and are treated under the heading of the latter (p. 35).

Attempts to Isolate Fat-soluble A.

Scarcely any attempt has been made as yet to isolate and identify this factor. The statement has been made that it may be removed from butter-fat by prolonged extraction with water, but recent work

does not confirm this (35). Certain facts may be interpreted as indicating that this accessory substance is a lipoid, but it has not been identified with any of the common representatives of that class of substance or with the free fatty acids (35).

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CHAPTER III

BERI-BERI AND SCURVY.

There is now a general consensus of opinion that beri-beri and scurvy are diseases of dietetic origin, caused by the absence from the diet of an essential accessory factor. Experimental work on animals and experience based upon human diet confirm each other in the view that the factors concerned in prevention of beri-beri and scurvy are distinct and have a different distribution among natural foodstuffs. In modern European life, under normal peace conditions, the risk of these deficiency diseases, although a real one where infants are concerned, may be regarded as non-existent for adults; the great variety of food taken ensures that an adequate amount of the preventive vitamines is regularly consumed. This fact will be appreciated later when the distribution of the factors preventing these diseases has been considered in detail. It is where extremes of climate limit the variety of available foodstuffs, as in the tropical or arctic zones, that a restricted diet may lead to outbreaks of deficiency disease. Thus beri-beri has proved to be mainly confined to rice-eating peoples in tfopical climates; scurvy is to be feared alike in tropical deserts or in arctic regions.

Under war conditions, both for civilians and soldiers, the situation is altered, even in civilized European countries. Owing to dislocation of transport and other causes, many articles of common consumption become scarce or absent and the diet becomes more and more restricted. Accurate knowledge as to the value of various articles of food in respect of the accessory factors contained in them becomes of the utmost importance. It is necessary to ensure that an amount of these be consumed in the diet sufficient to prevent the disturbance of metabolism which eventually leads to 'deficiency diseases'.

BERI-BERI.

Beri-beri is a disease characterized by severe nervous disorder occurring principally among rice-eating populations, e. g. in Japan, the Malay Peninsula, Dutch Indies, Philippine Islands, &c. It is a form of severe peripheral neuritis, and may appear in two well-marked forms. In one (dry type) there is great wasting, anaesthesia of the skin, and finally paralysis of the limbs; in the other (wet type) the most marked symptom is excessive oedema which may affect the trunk, limbs, and extremities. In severe cases the heart is usually involved, and death may occur suddenly from heart failure. The mortality is high.

This disease has, in the past, been regarded as a tropical disease confined to rice-eating populations. It is now recognized that this, though the best known example of endemic beri-beri, is only a special case of what will inevitably occur when the diet consists too exclusively of a cereal which has been impoverished by excessive milling. The researches of Eijkman, Grijns, and later of Braddon,

Fraser, and Stanton have shown that the disease does not occur among the East Indian and Malay rice-eaters when unmilled or home-milled rice is taken, and that it can be cured or prevented by restoring to 'polished'[1] rice the valuable constituents (germ or *embryo* and silverskin or *pericarp*) known as 'rice polishings', removed in the milling; see Fig. 11.

The discovery of avian polyneuritis was an important step towards the elucidation of the human disease. Eijkman (36), who in 1897 was medical officer to a prison in Java where beri-beri cases occurred, noticed that the poultry of the establishment showed paralytic symptoms strangely reminiscent of those of his patients, and died with extensive degeneration of the peripheral nerves.

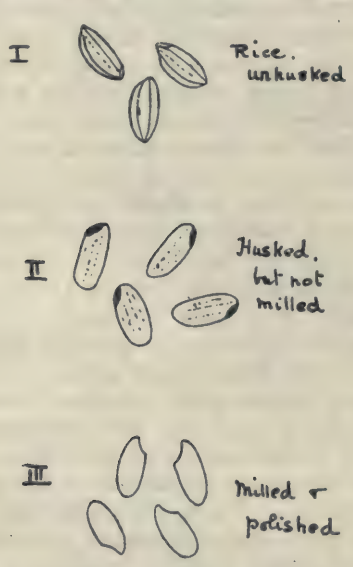

FIG. 11.[2] Showing the various stages in Milling of the Rice Grain. I. Rice grain in the natural condition, retaining the husk or enclosing glumes. II. After removal of the husk, but retaining the pericarp or 'silver-skin', and the embryo, which is shaded. III. After milling and polishing; both 'silver-skin' and embryo are removed and the grains are then 'polished' by rubbing with talc between sheepskins.

The fact that these fowls were largely fed upon the rice refuse of the institution was strongly suggestive of a dietetic origin for the disorder of the human inmates, a theory which, for many years previously, had attracted adherents. A careful study of polyneuritis gallinarum, as the disease was called by Eijkman, was published by Grijns (37) in 1901, but it was a matter for debate as to how far these experimental results could be applied to the human disease.

On further study, avian polyneuritis has been accepted as the physiological equivalent of human beri-beri, both in its etiology,

[1] Polished rice is a term which should strictly be applied to steam-milled rice after it has been subsequently polished with talc between sheepskins. The 'polishing' itself does not injure the rice dietetically, but the expression has been used loosely to indicate the whole process of preparation, and to include the milling in which cuticle and germ are removed.

[2] Reproduced from Chick and Hume, *Trans. Soc. Trop. Med. and Hyg.*, vol. x, 1917, p. 149, with the permission of that Society.

symptoms, and method of cure. By its discovery a method has been provided for determining experimentally which foodstuffs prevent beri-beri, and their relative value for this purpose. Experimental work with fowls and pigeons figures largely in the pioneer work by the above-mentioned observers, and in the later researches, among others of Schaumann, Vedder, Chamberlain, and Funk. (For complete bibliographies upon this subject see 38, 39, 39 a.)

McCarrison (39 b) has studied the loss of weight of various organs in pigeons fed with a diet which produces polyneuritis. With the exception of the adrenals the loss of various organs occurs much as in inanition but the adrenals increase to two or three times the weight of those of control birds. The adrenaline content of the enlarged glands appears to be increased proportionately to the size of the organ. McCarrison makes out a relationship between the hypertrophy of the adrenals and the occurrence of hydropericardium in his experimental birds. He regards the excess of liquid as the equivalent of 'wet beri-beri' in man, and develops a theory of the cause of oedema in beri-beri on the basis of excessive adrenaline production.

Distribution among Foodstuffs of the Anti-beri-beri or Anti-neuritic Factor.

The work of the early investigators in this field yielded much valuable information upon this point, but the distribution of the anti-beri-beri vitamine was first studied systematically by Cooper (40) working at the Lister Institute. In response to war-time needs this work has since been rendered more complete by other workers in the same Institute (Chick and Hume, 41, 46).

The methods adopted were substantially the same throughout; pigeons were used as experimental animals, and the experiments were of two general types—preventive and curative.

In the *preventive* experiments estimate was made of the daily ration of the particular foodstuff necessary to prevent polyneuritis when added to a vitamine-free diet consisting of highly milled and polished rice. Feeding was artificial, and in the absence of an adequate supply of vitamine in the supplementary foodstuff the birds developed severe symptoms in from 15–25 days, with paralysis of legs and wings, leading to complete helplessness and death within 24–48 hours. Protection was reckoned to be successfully accomplished if the birds showed no symptoms after a period of 50–60 days.

In *curative* experiments a series of birds was fed with polished rice until severe symptoms were observed. The foodstuff to be tested was then given by the mouth in varying doses to separate birds and determination made of the dose required to cure the symptoms. One of the most striking features of avian polyneuritis is the dramatic swiftness with which a bird will recover from complete helplessness. It is not unusual to see normal gait and flight restored within a few hours after the adequate amount of vitamine has been administered.

It is sometimes necessary to concentrate the factor contained in the foodstuff in order to get the requisite amount absorbed in time. In such cases a weighed quantity of the air-dried material is shaken up with alcohol in the cold, the alcoholic extract evaporated

Table II. *Preventive Experiments.*

Minimum Daily Ration which must be added to a Diet of Polished Rice to prevent Onset of Polyneuritis (Beri-beri) in a Pigeon of 300 to 400 grm. Weight.

Substance.	*Daily Ration.*		*Observer.*	*Notes on Results.*
	Natural Foodstuff. (*grm.*)	*Dry Weight.* (*grm.*)		
Wheat germ, sample R.I., free from bran	1·5	1·3	Chick and Hume (46)	Complete protection.
Wheat bran, sample R.I., free from germ	More than 1·5	More than 1·4	,, ,,	No protection with 1·5 grm. daily. Polyneuritis occurred in same time as in control birds.
,, ,, ,,	More than 2·5	More than 2·2	,, ,,	Small degree of protection. Mean time of onset delayed about 2 weeks.
Yeast extract, commercial sample A	0·5	0·35	,, ,,	Protection not secured in all cases.
	1·0	0·7	,, ,,	Protection.
Pressed yeast	2·5	0·5	Cooper (40)	,,
Lentils	—	3·0	,, ,,	,,
Barley, unhusked	3·7	3·2	,, ,,	,,
Barley, husked	5·0	4·5	,, ,,	,,
Egg-yolk	3	1·5	,, ,,	,,
Beef-muscle	20	5·0	,, ,,	,,
Ox heart-muscle	5	1·7	,, ,,	,,
Ox brain	6	1·2	,, ,,	,,
Ox liver	3	0·9	,, ,,	,,
Sheep brain	12	2·5	,, ,,	,,
Fish-muscle	More than 10	More than 2	,, ,,	,,
Cheese	More than 8	More than 5·6	,, ,,	Protection not secured with these amounts.
Cow's milk	More than 35	More than 3·5	,, ,,	Protection not secured with these amounts.

Table III. *Curative Experiments.*

Minimum Amounts of Foodstuffs required to cure Polyneuritis (Beri-beri) in a Pigeon 300 to 400 grm. Weight. The Doses are reckoned in Terms of the Original Foodstuffs.

Substance.	*Preparation of curative material.*	*Amount of dose given.* *In terms of the natural foodstuff.* (*grm.*)	*In terms of dry weight* 100–110° C. (*grm.*)	*Result.*	*Observer.*
Wheat germ					
Commercial sample, cooked .	Extracted with alcohol	8	6·9	Improvement	Chick and Hume (46)
		12	10·3	Sometimes cures	,, ,,
		16	13·8	Cure	,, ,,
Sample A 'picked', uncooked .	,, ,,	10	8·7	Incomplete cure	,, ,,
		15	13·0	Complete cure	,, ,,
Sample B 'picked', uncooked .	Unextracted	1·0	0·9	Sometimes cures	,, ,,
		2·5	2·2	Cure	,, ,,
*Maize germ	,,	1·0 to 3·0	—	,,	,, ,,
*Rice germ	,,	0·5 to 1·0	—	,,	,, ,,
Wheat bran					
Stone ground, not free from germ	,,	5·0	—	Sometimes cures	,, ,,
Roller milled, free from germ .	,,	3·0	—	No cure	,, ,,
		5·0	—	Sometimes cures	,, ,,
Rice bran, containing germ . .	,,	6·0	—	No cure	,, ,,
		10·0	—	Complete cure	,, ,,
Turbot fish-roe (hard) . .	Extracted with alcohol	35	10	Cure almost complete	,, ,,
		70	20	Cure complete	,, ,,
		140	40	,, ,,	,, ,,
Egg-yolk	,, ,,	60=4 yolks	30	Cure	Cooper (40)
Dried whole egg, commercial samp. 1	Unextracted	40=about 4 yolks	38	,,	Chick and Hume (46)
,, ,, ,, ,,	,,	30= ,, 3 ,,	29	,,	,, ,,
,, ,, ,, samp. 2	,,	20= ,, 2 ,,	—	,,	,, ,,
Malt extract, 1st sample . .	,,	5	4·2	,,	Cooper (40)
,, 2nd sample . .	,,	7	5·1	,,	,, ,,
,, 3rd sample . .	,,	10	9·2	No cure	,, ,,
Meat extract, commercial sample 1	,,	3	2·8	No improvement, bird died	Chick and Hume (46

* Picked out from the unmilled grain by hand in the laboratory.

Curative Experiments (continued).

Substance.	*Preparation of curative material.*	*Amount of dose given.*		*Result.*	*Observer.*
		In terms of the natural foodstuff.	*In terms of dry weight* 100–110° C.		
		(*grm.*)	(*grm.*)		
Meat extract, commercial sample 1	Unextracted	6·5	5·2	No improvement, bird died	Chick and Hume (46)
Raw beef	Extracted with alcohol	140	30 approx.	Cure	Cooper (40)
'Maconochie' ration . . .	,, ,,	440	106	Cure incomplete	Chick and Hume (46)
'New ration' roast beef tinned (submitted for examination by the Dept. of Hygiene, R.A.M. College, on June 19, 1916)	,, ,,	350	112	Very slight improvement, no cure	,, ,,
Dried peas	,, ,,	30	26	Incomplete cure	,, ,,
,, ,, ...	Unextracted	40	35	Complete cure	,, ,,
		10	9	Cure	,, ,,
Pea flour, kilned . . .	Extracted with alcohol	80	—	Slight improvement, no cure	,, ,,
Dried lentils	,, ,,	20	18	Cure	Cooper (40)
,, vegetables, commercial sample	,, ,,	40	35	,,	Chick and Hume (46)
'Spring greens' .	,, ,,	120	105	Complete cure	,, ,,
Potatoes, peelings . . .	,, ,,	180	36	Died	,, ,,
,, ,, ...	,, ,,	630	126	Improvement, no cure	,, ,,
,, insides . . .	,, ,,	200	40	Incomplete cure	,, ,,
,, ,, . . .	,, ,,	350	70	Cure	,, ,,
Pressed yeast	Autolysed	2·0	0·4	Cured slowly	,, ,,
,, ,, ...	,,	3·0 to 6·0	0·6 to 1·2	Cure	Cooper (40)
Yeast extract, commercial sample A	Unextracted	1·5 to 2·0	1·0 to 1·3	Complete cure	Chick and Hume (46)
Dried fruits, currant . . .	Extracted with alcohol	60	47	No improvement	,, ,,
,, ,, ,, . . .	,, ,,	19	16	,,	,, ,,
,, ,, dates . . .	,, ,,	26	21	Slight improvement, death delayed	,, ,,
,, ,, ,, . . .	,, ,,	26	21	Slight temporary improvement	,, ,,
Proprietary article, dry powder, stated to contain vitamines in a concentrated form	Unextracted	12	—	No improvement, died	,, ,,
		31	—	Temporary improvement, no cure	,, ,,

to dryness under reduced pressure and the residue taken up in a measured quantity of water. Various doses of this watery solution are then given to a series of birds with severe symptoms in order to determine the minimum amount necessary to effect a cure. There is a considerable mechanical loss of the anti-neuritic factor in the process of extraction, and in making any comparison of values it is, therefore, necessary to make sure that the curative materials, as employed, are strictly comparable (see above, Table III; in the case of dry peas the curative dose of natural material is 10 grm., whilst that of the alcohol extract is the equivalent of 40 grm.; in the case of wheat germ the amounts were 1 to 2·5 grm. and 16 grm. respectively).

The results of the experiments on the distribution of the anti-beri-beri factor by Cooper and Chick and Hume are summarized in Tables II and III. The results in Table II, based on preventive experiments, must be considered the more valuable. If, however, the researches had been limited to this type of experiment, their scope must of necessity have been very much curtailed. In Table IV is shown the approximate relative value of some of the more potent of the foodstuffs investigated.

TABLE IV. *Showing approximately relative values of the more important foodstuffs (weight for weight) in natural condition for prevention of beri-beri, compiled from results in Tables II and III.* (Value of wheat germ taken as equal to 100.)

Substance.	*Value.*	*Approximate water content.* *Per cent.*
Wheat germ	100	10 to 13
Wheat bran	25	10 to 13
Rice germ	200	10 to 13
Yeast (pressed)	60	70
Peas (dried)	40	12
Lentils	80	
Egg yolk	50	70
Ox liver	50	70
Beef muscle	11	75
Potatoes	4·3	80

It will be seen that the anti-beri-beri or anti-neuritic factor is widespread; it was detected in almost all the natural foodstuffs examined. Its presence in yeast, the only unicellular organism studied, is significant of the universality of its distribution. Its principal sources proved to be the seeds of plants and eggs of birds, thus following very closely the distribution of the water-soluble growth factor and suggesting strongly the probable identity of the two (see p. 35).

Cereals. The most important practical result emerging from this work is the fact that one of the chief sources of anti-beri-beri vitamine should be found in the cereals and edible pulses. In case of the cereals an interesting differentiation has been established (Chick and Hume 41) between the different constituents of the grain, the largest deposit of the anti-neuritic factor being found in the embryo, or germ, the bran (*pericarp* and *aleurone layer*) coming next in order of importance (see Fig. 12*a*). The endosperm,

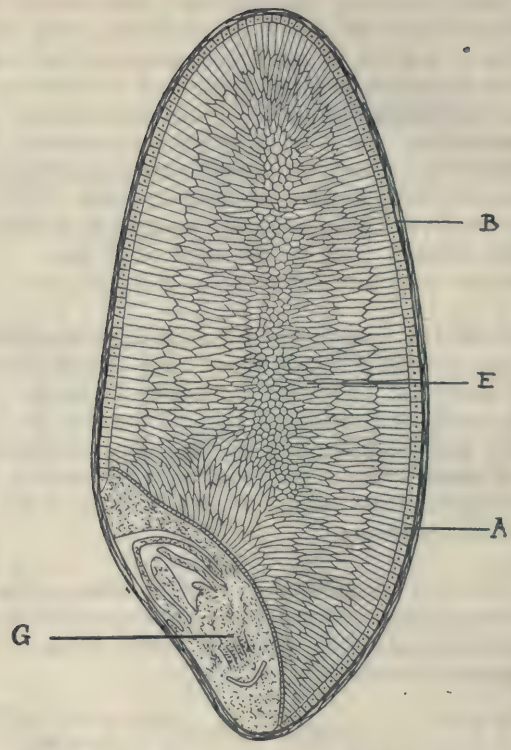

FIG. 12 *a*.[1] Diagram of a longitudinal section through a grain of wheat, showing: *B*, Pericarp, forming the branny envelope. *A*, Aleurone layer of cells forming the outermost layer of the endosperm removed with the pericarp during milling. *E*, Parenchymatous cells of the endosperm. *G*, Embryo or germ.

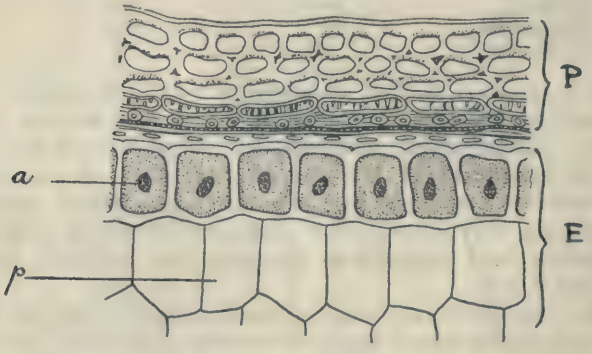

FIG. 12 *b*.[1] Cross-section through the branny envelope and outer portion of the endosperm of wheat grain, showing: *P*, the pericarp; *E*, endosperm, consisting of *a*, layer of aleurone cells and *p*, parenchymatous cells.

[1] Reproduced, with the permission of the Controller of H.M. Stationery Office, from Figs. 1 and 2 in Dr. J. M. HAMILL'S 'Report to the Local Government Board on the nutritive value of bread made from different varieties of wheat flour', 1911.

especially when deprived of the *aleurone layer* (as is customary in the preparation of white wheaten flour or 'polished' rice), is deficient in the anti-neuritic factor, and if employed too exclusively as a diet will occasion polyneuritis in birds or beri-beri in man [see also Edie and Simpson (42)].

The experiments were made chiefly with rice and wheat, but there is confirmatory evidence in case of maize and rye, and no reasonable doubt exists that, as a rule among cereals, the anti-beri-beri factor is chiefly concentrated in the embryo.

The potency of the cereal embryo in curing avian polyneuritis was found to be very great. Chick and Hume found that 2·5 grm. (and occasionally 1 grm.) wheat germ was sufficient to cure a pigeon showing fully developed symptoms; in case of rice and maize embryo, cures were obtained with amounts varying from 0·5 to 1 grm. and from 1 to 3 grm. respectively.

The prevailing opinion, based on observation of rice-eating populations consuming decorticated rice, has located the principal deposits of the substance preventing beri-beri in the cuticle of the husked grain, in the outer layer (*aleurone layer*) of endosperm, which is removed during steam milling. This view was based upon the connexion between beri-beri and decorticated rice, and the prevention or cure of the disease effected when rice bran or its extracts were added to the diet. A truer explanation recognizes the richer source of the vitamine to be in the germ (*embryo*) of the cereal removed with the bran. In the case of wheat and rice the germ, weight for weight, was found to be respectively five times and ten times as potent as the bran in the cure of avian polyneuritis.

Pulses. The foodstuffs next in importance to the cereals in prevention of beri-beri are the pulses. Grijns (37) and others have demonstrated the value of certain beans ('katjang-idjo'—*Phaseolus radiatus*) in prevention and cure of avian polyneuritis or human beri-beri. Dry peas and lentils were found to occupy a high place in the series of foodstuffs collected in Table IV.

Eggs are also valuable sources of the anti-beri-beri factor, and this property was found unimpaired in two samples of dried eggs examined. These large deposits of anti-neuritic factor in the seeds of plants and eggs of animals (birds and fishes) suggest a due provision made for the wants of the young offspring during the early period of life. Vedder (43, p. 61) mentions the unusual prevalence of beri-beri among pregnant women in countries where the disease is endemic.

Yeast is rich in this accessory factor and is unharmed in this respect by autolysis and extraction. Yeast extract A in Tables II and III was a commercial preparation (*marmite*) largely used as a substitute for meat extract in preparation of soup cubes, &c.

Meat is comparatively deficient in this factor, and large quantities were required for curing or preventing pigeon polyneuritis. This was specially true of muscle fibre which was less valuable than heart, liver, or brain.

In case of preventive experiments with fish, milk, and cheese protection was not obtained with the comparatively large amounts administered daily (experiments by Cooper, Table II).

Resistance to Drying, Heat, &c., and other Properties of the Anti-Beri-Beri or Anti-Neuritic Factor.

The anti-beri-beri factor withstands desiccation for long periods of time, as may be appreciated from the fact that its principal sources are found in dry foodstuffs.

Its resistance to heat is also considerable. Many isolated observations upon this point have appeared in the literature during the last twenty years, and most observers have not detected destruction of the anti-beri-beri vitamine after exposure to 100° C. Grijns (37) found that 1–2 hours' exposure to 120° C. destroyed the anti-beri-beri properties of unmilled rice, katjang-idjo beans, and buffalo meat. Eijkman (44) did not succeed in destroying the protective value of horseflesh by heating for two hours at 120° C. Holst (45) traced loss of anti-neuritic properties in beef heated to 110° C. for half an hour, but in the case of dried peas or unpeeled barley kept at 115° C. for thirty minutes, no loss of anti-neuritic power was detected.

Recently Chick and Hume (46) have made a series of systematic experiments upon this point, with (*a*) wheat germ (natural condition, 15 per cent. moisture), and (*b*) yeast extract (containing 65 per cent. water) exposed to temperatures from 100° C. to 124° C. in a steam autoclave. The results obtained are summarized in Table V, from which it will be seen that destruction of anti-beri-beri vitamine takes place very slowly at 100° C., but is much more rapid in the neighbourhood of 120° C.

Table V.

Influence of Exposure to high Temperatures upon the Anti-Beri-Beri Vitamine contained (a) *in Wheat Embryo and* (b) *in Yeast Extract.*

Substance.	*Temperature.* ° *C.*	*Time.* *Min.*	*Minimum amount in grm. required to cure a pigeon (300 to 400 grm.) suffering from acute polyneuritis.*
Wheat embryo, water content 11 to 14 %	Unheated	Control	1·0 to 2·5
	98 to 103	120	2·5
	100 to 117	40	5·0
	118 to 124	120	10·0 did not cure
Yeast extract A, water content 65 %	Unheated	Control	1·5 to 2·0
	100	60	2·0 to 3·0
	122	60	2·5 to 3·0
	122	120	about 5·0

The practical conclusion to be drawn from this result is that in the baking of bread or biscuit, during which process the interior of the material does not rise above 100° C., no serious diminution in anti-beri-beri vitamine need be apprehended. This proved to be true in the case of pigeons, which throve well upon a sole diet of wheaten biscuit made from wholemeal flour. In preserving and canning foodstuffs, however, the temperatures employed are frequently much higher than 100° C., and it is safe to regard tinned foods of all descriptions with but few exceptions as vitamine-free.

This is well seen in Table III, where the curative dose of raw meat for pigeons is given as the equivalent of 30 grams dry weight, and where, in two instances of tinned meats, no cures were obtained with amounts equivalent to 106 and 112 grams dry weight respectively.

The anti-neuritic accessory substance can be extracted by alcohol from most of the foodstuffs in which it is found ; it is also soluble in water. It is not, as a general rule, extracted by ether or other fat-solvents, although Cooper (40) was able to detect distinct anti-neuritic properties in the ether extract from egg-yolk. It is probable that, as in the case of the fat-soluble accessory factor, the ' solubility ' of this vitamine is variable, and depends on the character of the material in which it is deposited. The anti-neuritic accessory substance is readily adsorbed from solutions upon the surface of various adsorbents, e. g. animal charcoal (Chamberlain and Vedder, 47), fuller's earth (Seidell, 48), colloidal ferric hydroxide (Harden and Zilva, 49). The anti-neuritic factor may be almost completely removed by one or another of these adsorbents, and there is no doubt that this property offers an explanation of the mistaken belief that the factor itself had been successfully isolated (p. 37).

Probable Identity of the Water-soluble B and Anti-neuritic Factors.

It is now very widely believed that the water-soluble B and anti-neuritic factors are identical (see Drummond, 65). This conclusion is based in the first place upon the fact that when the distribution and properties of the water-soluble *B* factor, marked out by the use of rats, are compared with those of the anti-neuritic factor, established by experiments on pigeons, the closest possible agreement is found to exist between the two sets of results (see Tables VI and VII). Additional evidence is afforded by a comparison of the symptoms which are developed by rats and pigeons suffering from deficiencies of these factors. In both cases these point to the presence of a peripheral neuritis, and this is confirmed by the histological examination of the nerves.

Table VI.

Table showing Identity of Distribution of the Water-soluble B and the Anti-neuritic Factor.

Foodstuff.	*Value as source of Water-Soluble B Factor (as shown by experiments on rats).*	*Value as source of Anti-beri-beri or Anti-neuritic Factor (as shown by experiments on birds).*
Rice germ	+ + +	+ + + +
Wheat germ	+ + +	+ + +
Linseed	+ +	
Lentils		+ + +
Yeast	+ + +	+ + +
Egg-yolk	+ +	+ + +
Ox liver	+ +	+ + +
Wheat bran	+	+ +
Fresh peas	+	
Dried peas		+ +
Fresh carrots	+	
Dried carrots	+	
Meat muscle	+	+
Milk	+	Slight
Potatoes	+	+
Meat extract	0	0
Meat tinned		v. slight
White bread or flour	0	0
Polished rice	0	0

Table VII.

Table showing Properties of Water-soluble B Factor and Anti-neuritic (Anti-Beri-Beri) Factor, as determined by experiments on growth of Rats and prevention of Avian Polyneuritis respectively:

		Water-soluble B Factor.	*Anti-neuritic Factor.*
Solubility.	Water.	Very soluble.	Very soluble.
	Alcohol, dilute.	Very soluble.	Very soluble.
	Alcohol, absolute.	Insoluble.	Insoluble.
	Ether.	Insoluble.	Usually insoluble, but can be extracted with ether from fatty materials such as egg-yolk.
	Chloroform.	Insoluble.	
	Benzene.	Insoluble	
Stability.	Heat.	Stable at 100° C., destroyed rapidly at 120° C. (in neutral or acid solution).	Destroyed very slowly at temperatures below 100° C., destroyed more rapidly at 110 to 120° C.
	Drying.	Stable.	Stable.
	Acids, hot dilute.	Moderately stable.	Stable.
	cold „	Stable.	Stable.
	Alkalies, hot dilute.	Rapidly destroyed.	
	cold „	Stable.	
Behaviour on dialysis.		Passes through parchment membrane.	Passes through parchment membrane.
Adsorption.		Adsorbed from acid or neutral solution by fuller's earth, charcoal, &c.	Adsorbed from neutral solutions by fuller's earth, colloidal ferric hydroxide, animal charcoal, &c.

Attempts to Isolate the Water-soluble Accessory Substance or Anti-neuritic Vitamine.

A large number of attempts have been made to obtain this accessory substance in a pure condition, but, as they have all hitherto failed, it is only necessary to review the more important investigations. Cooper and Funk (50) found that the curative substance present in an alcoholic extract of rice polishings was precipitated by phosphotungstic acid, and that this method of preparation yielded a fraction which was ' extraordinarily potent ', and free from proteins, carbohydrates, and phosphorus. Funk carried the investigation further (51), and fractionated the substances precipitated by phosphotungstic acid by means of precipitations with silver nitrate, following the usual method employed for separating certain nitrogenous bases. From the silver nitrate-baryta fraction he obtained a crystalline substance melting at 233° C., to which he gave the formula $C_{17}H_{20}O_7N_2$. This substance was curative for pigeons. This process of separation was applied to yeast and certain other foodstuffs (Funk, 52), and led to the isolation of a substance possessing similar properties.

Funk considered that the chemical properties of the curative substance placed it in the class of pyrimidine bases.

The substances isolated by Funk do not, however, appear to be the active substance in a pure condition. Rather does it seem that they are nitrogenous bases contaminated with traces of the accessory factor (Barger, 53 ; Drummond and Funk, 54). Another attempt to isolate the factor was that made by Susuki, Shinamura, and Odake (55), who claimed to have prepared the anti-neuritic substance, which they termed oryzanin, in the form of a crystalline picrate. Drummond and Funk (54) failed to confirm their results.

Edie, Evans, Moore, Simpson, and Webster (56) obtained an active fraction from yeast, from which they precipitated a crystalline basic substance belonging to the pyrimidine group. This they termed torulin.

The precipitation of the active substance by silver nitrate and baryta has been confirmed by Cooper (57) and by Vedder and Williams (78) and Williams and Saleeby (79). Further, Williams and Seidell (60) prepared a sample of adenine from yeast, and found that this had anti-neuritic properties, but lost them on keeping. They attributed this change to isomerisation, but their results have not been confirmed by other workers (Voegtlin and White, 61 ; Harden and Zilva, 62).

Quite recently Abderhalden, who on more than one occasion has vigorously attacked the vitamine theory, has published a paper with Schaumann (63), in which they report attempts to isolate the accessory substance of yeast. This long and obscure paper marks little or no advance in this field ; no active substances were obtained in a pure condition, the previous work of many investigators is ignored, and the subject is still further confused by the introduction of new names (eutonin and nutramine) for the accessory substances.

Hofmeister, in his recent review of the vitamines (64), states that he has isolated from a very active solution a substance belonging to the pyrimidine series. It yields a crystalline hydrochloride and double salt with gold chloride, and he has given it the formula

$C_5H_{11}NO_2$. The details of his work do not appear to have been published as yet. Drummond (65) has made a study of the water-soluble accessory factor in yeast extract, and has suggested that many of the results obtained by previous investigators may be explained by the readiness with which this substance is carried down in the adsorbed condition by precipitates.

SCURVY.

Scurvy has for many centuries been regarded as a disease due to dietetic errors, and rightly so. It was common knowledge in olden times, especially among seafaring folk, that scurvy occurred after deprivation for long periods of fresh foodstuffs, and that it could be prevented and rapidly cured when fresh vegetables and fruits were available. Thus Bachstrom in 1734 (*Observationes circa scorbutum ; eiusque indolem, causas, signa et curam*) wrote as follows :

' From want of proper attention to the history of the scurvy, its causes have been generally, though wrongfully, supposed to be cold in northern climates, sea-air, the use of salt meats, etc., whereas this evil is solely owing to a total abstinence from fresh vegetable food and greens ; which is alone the true primary cause of the disease. And where persons, either through neglect or necessity, do refrain for a considerable time from eating the fresh fruits of the earth, and greens, no age, no climate or soil are exempted from its attack. Other secondary causes may likewise concur, but recent vegetables are found alone effectual to preserve the body from this malady ; and most speedily to cure it, even in a few days, when the case is not rendered desperate by the patients' being dropsical or consumptive ' (translation given by Lind, 66, p. 394).

Other theories of the etiology of scurvy dating from more recent times include, firstly, that of bacterial origin frequently advanced from different sources, and secondly, the suggestion that the cause is chronic poisoning from putrefying meat or fish. Many adherents of the former theory (e. g. Coplans, 67 ; Jackson and Moody, 68) have brought forward some experimental support for their view, the explanation being doubtless that any animal in a scorbutic condition, due to dietetic deficiency, will be a ready prey to a secondary infection. The second view, first advanced by Torup (quoted by Jackson and Harley, 69) and supported by the work of Jackson and Harley (69), has received wide acceptance in recent times, especially among the leaders of some Arctic expeditions, with the result that the nature of the stores taken and diet arranged was modified accordingly. There is, however, little, if any, support for this view in the final experience of the expeditions, and the interpretation placed by Jackson and Harley upon their experimental results has not been accepted by their scientific colleagues (Holst and Frölich, 70, p. 42).

It is convenient in this place to allude to the most recent attempt to explain scurvy on lines other than that of a deficiency disease, although the theory refers to the experimental scurvy of guinea-pigs rather than to human scurvy. McCollum and his co-workers (71, 72, and 73) consider guinea-pig scurvy to be caused by chronic constipation brought on by an unsuitable, rather than a defective,

diet. Their view is based mainly upon the different results obtained in guinea-pigs and rats, respectively, by a diet of milk and grain; the guinea-pigs were found frequently to die of 'scurvy', while the rats grew in a normal manner. These workers, therefore, consider that this diet contains adequate nutritive constituents for both types, so that the failure on the part of the guinea-pig must be referred to a defect in the physical properties of the diet, i. e. to lack of 'roughage'. In the light of the recent experimental work on milk detailed on p. 45, it is evident that the discrepancy can be explained by the low value of milk as an anti-scorbutic foodstuff, coupled with a difference in the requirements of these two animals as regards the *amount* of anti-scorbutic vitamine necessary for the maintenance of health.

McCollum and his colleagues confine their interpretation of scurvy to the experimental disease produced in guinea-pigs, but this disorder has been accepted on convincing evidence as the physiological equivalent of human scurvy by all other workers in this field. It is evident, therefore, that the identity of the two disorders must be denied or that the theory of McCollum must be applied also to human scurvy. The evidence against such a view is overwhelming, and as regards the guinea-pig disease, McCollum's views have been combated both in England and America in a series of publications (74, 75, 76, 77, 78, 79, and 80) showing how the experimental results of McCollum and his colleagues can reasonably be interpreted to give full support to the vitamine hypothesis.[1]

Systematic experimental work on scurvy dates from the early part of the present century, when Professor Axel Holst and his colleagues at the University of Christiania made a careful study of guinea-pig scurvy, its etiology, symptoms, and methods of prevention and cure, and decided that it offered a complete analogy with the human disease. One or two centuries previously, when scurvy was an everyday occurrence and the common dread of all seamen, rough 'experiments' were from time to time made upon the human material so unfortunately provided. Thus Lind, in the middle of the eighteenth century, published the following account of an 'experiment', which expresses, as the result of his own careful observation, what became the common knowledge of his own day.

'On the 20th May, 1747, I took twelve patients in the scurvy, on board the "Salisbury" at sea. Their cases were as similar as I could have them. They all in general had putrid gums, the spots and lassitude, with weakness of their knees. They lay together in one place, being a proper apartment for the sick in the fore-hold; and had one diet common to all, viz. water-gruel sweetened with sugar in the morning, fresh mutton-broth often times for dinner; at other times light puddings; boiled biscuit with sugar, etc., and for supper, barley and raisons, rice and currants, sago and wine, or the like.

'Two of these were ordered each a quart of cyder a day. Two others took twenty-five drops of *elixir vitriol*, three times a day,

[1] According to a private communication received from Professor McCollum he has been convinced by these arguments and now regards guinea-pig scurvy as a deficiency disease.

upon an empty stomach; using a gargle strongly acidulated with it for their mouths. Two others took two spoonfulls of vinegar three times a day upon an empty stomach; having their gruels and their other food well acidulated with it, as also the gargle for their mouths. Two of the worst patients, with the tendons under the ham rigid (a symptom none of the rest had) were put under a course of sea-water. Of this they drank half a pint every day, and sometimes more or less, as it operated by way of gentle physic. Two others had each two oranges and one lemon given them every day. These they ate with greediness, at different times upon an empty stomach. They continued but six days under this course, having consumed the quantity that could be spared. The two remaining patients took the bigness of a nutmeg three times a day of an electary recommended by an hospital-surgeon, made of garlic, mustard-seed, *rad. raphan*, balsam of *Peru*, and gum myrrh; using for common drink, barley-water well acidulated with tamarinds; by a decoction of which, with the addition of *cremor-tartar*, they were gently purged three or four times during the course.

'The consequence was, that the most sudden and visible good effects were perceived from the use of the oranges and lemons; one of those who had taken them being at the end of six days fit for duty. The spots were not indeed quite off his body, nor his gums sound; but without any other medicine, than a gargarism of *elixir vitriol*, he became quite healthy before we came into Plymouth, which was on the 16th. June. The other was the best recovered of any in his condition; and being now deemed pretty well, was appointed nurse to the rest of the sick.

'Next to the oranges, I thought the cyder had the best effects. It was indeed not very sound, being inclinable to be aigre or pricked. However, those who had taken it were in a fairer way of recovery than the others at the end of the fortnight, which was the length of the time all these different courses were continued, except the oranges. The putrefaction of their gums, but especially their lassitude and weakness, were somewhat abated, and their appetite increased by it.

'As to the *elixir of vitriol*, I observed that the mouths of those who had used it by way of gargarism, were in a much cleaner and better condition than many of the rest, especially those who used the vinegar; but perceived otherwise no good effects from its internal use upon the other symptoms. I indeed never had a great opinion of the efficacy of this medicine in the scurvy, since our longest cruise in the "Salisbury" from the 10th. August to the 28th. October, 1746; when we had but one scorbutic case in the ship. The patient was a marine (one Walsh), who after recovering from a quotidian ague in the latter end of September, had taken the *elixir vitriol*, by way of a restorative for three weeks; and yet at length contracted the disease, while under a course of medicine recommended for its prevention.

'There was no remarkable alteration upon those who took the electary and tamarind decoction, the sea-water, or vinegar, upon comparing their condition, at the end of the fortnight, with others who had taken nothing but a little lenitive electary and *cremor-*

tartar, at times, in order to keep their belly open ; or a gentle pectoral in the evening, for relief of their breast ' (Lind, 66, p. 56).

Holst and Frölich (70, 81) showed that scurvy could be induced in guinea-pigs by removing the greenstuff from the ordinary diet of grain and cabbage leaves, and by giving a diet consisting of grain and water only. It proved immaterial what grain was used, and the experiments included uncooked maize, oats, barley, and rice, as well as wheat and rye in the form of bread. In all cases this diet caused severe scurvy from which the animals died in from twenty to forty days, showing the haemorrhages and bony changes characteristic of the disease. By supplementing the scurvy diet with fresh vegetables, fruits and fruit juices, these observers were able to maintain the animals in health. For example, scurvy was prevented by a daily ration of 30 grm. of fresh raw cabbage, dandelion leaves, sorrel, carrot or cranberries. When these anti-scorbutic materials were heated, their value was reduced by an amount depending on the temperature and time of heating ; after heating at 100° C. for one hour (and in some cases for half an hour) the same ration was found inadequate to prevent scurvy. A similar result was obtained with dried materials ; rations equivalent to 30 grm. of raw carrot, dandelion leaves or cabbage leaves failed to prevent scurvy when in the dried condition, and equally disappointing results were obtained with dried potatoes.

The anti-scurvy substance originally present in the expressed juices of vegetables suffered a similar fate rapidly on keeping, but the expressed juices of acid materials, e. g., lemons, raspberries or sorrel leaves were found to be more stable in this respect.

A further result of first-class importance was obtained by Fürst (82) also working in Professor Holst's laboratory. He found that, whereas dry cereals or pulses, e. g., oats, barley, lentils, peas, beans, were unable to prevent scurvy in the dry condition, if soaked in water and allowed to begin germination for 2–3 days they acquired anti-scurvy properties. This result has recently been confirmed by Chick and Hume (83), and Chick and Delf (84) as regards pulses. In the case of cereals it has been called in question by Weil, Mouriquand, and Péronnet (85). The published account of their experiments is not very detailed, and it does not seem impossible that they might be interpreted in the opposite sense to that adopted by the authors themselves.

From the researches of Holst and his colleagues we are enabled to make the following generalization. The anti-scorbutic accessory factor is found in nature associated with living tissues in which metabolic processes are still proceeding. When these active processes cease or are greatly reduced, as in seeds, or when the tissues are destroyed, as in drying or heating, the anti-scurvy ' vitamine ' also disappears. In the case of seeds it is created anew during germination. The distribution of this factor thus presents a marked contrast with that of the anti-beri-beri factor, of which one of the principal sources is found in dry seeds.

The researches of Holst and his co-workers have been continued during the last three years by a group of workers at the Lister Institute, with the aim of making a more complete survey of the commoner

foodstuffs and assigning to each some quantitative value as regards anti-scurvy properties. Although the investigation is still incomplete, many results have already been published owing to the urgent need

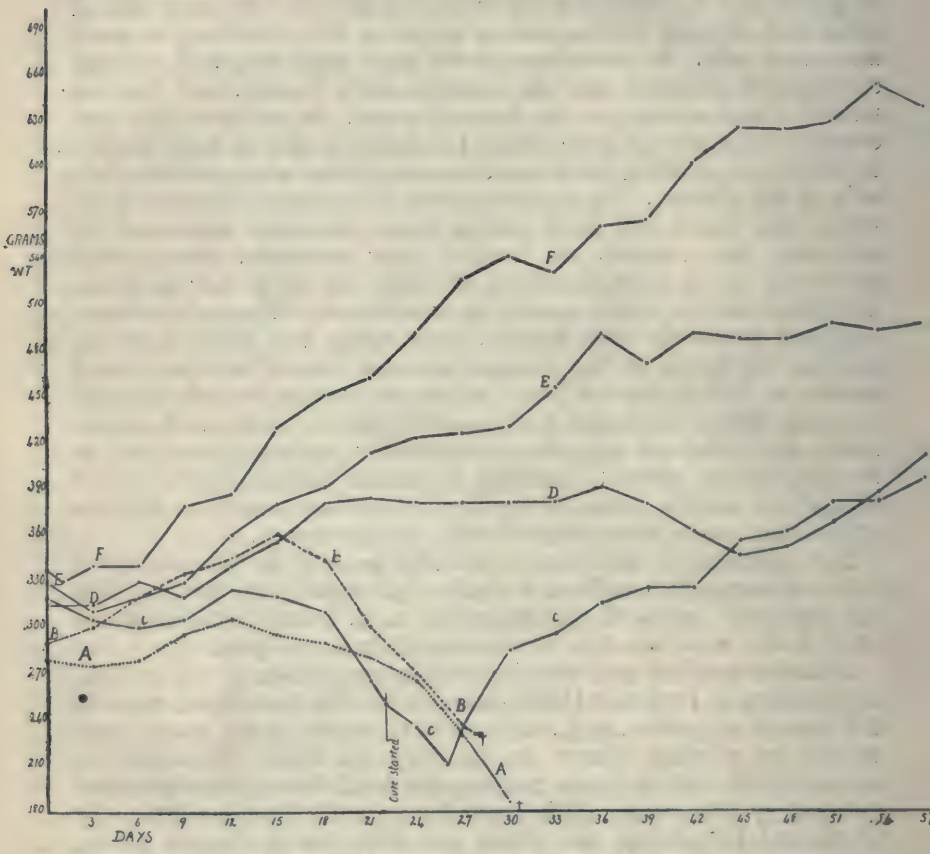

FIG. 13.[1] Weight Charts of five typical experiments with guinea-pigs showing the anti-scorbutic value of orange juice and fresh cabbage leaves :

CURVE A. Typical scurvy on a diet of oats, bran and water.

CURVE B. Typical scurvy on a diet of oats, bran and sterilized milk (heated to 120° C. for one hour in the autoclave).

CURVE C. Typical scurvy on a diet of oats, bran and water, cured by addition of orange juice and autoclaved milk to the diet on the twenty-second day, when the symptoms were well marked.

CURVE D. Weight chart showing influence of 5 c.c. fresh orange juice daily added to the ' scurvy ' diet ; autoclaved milk added to the diet on the fifty-sixth day.

CURVE E. Normal weight chart on a diet of oats, bran and cabbage leaves (30 grm. daily).

CURVE F. Weight chart on diet of oats, bran, autoclaved milk and 3 c.c. fresh orange juice daily ; specially favourable circumstances—warm weather, &c.

for such knowledge presented by war-time conditions (Chick and Hume, 83 ; Chick, Hume, Skelton, and Smith, 86 ; Chick and Rhodes, 87 ; Delf, 88 ; Delf and Skelton, 89). To these workers and to other members of the staff at present engaged in this research we

[1] Reproduced from the figure in the *Transactions of the Society of Tropical Medicine and Hygiene* with the permission of that Society ; Chick and Hume, vol. x, 1917, p. 152.

Fig. 14

are indebted for much unpublished information, and for permission to incorporate it in the following paragraphs.

Young, growing guinea-pigs (350 grm. weight) have been employed for the work, the methods used being in essential those of Holst and his colleagues. The principal modification has been the substitution of water in the scurvy diet of grain and water by a daily ration of about 60 c.c. strongly heated (autoclaved at 120° C. for one hour) milk. This addition of milk very greatly improves the general condition of the experimental animals, although the onset of scurvy is not thereby seriously influenced. Normal growth takes place for the first 15 to 20 days of the experiment in spite of absence of anti-scorbutic material; with onset of scurvy symptoms, about the twentieth day, the weight of the animal begins to decline, death from acute scurvy ensuing about the thirtieth to fortieth day. The general type of the weight curve on a scurvy diet with and without the addition of autoclaved milk is well seen in Fig. 13 by observing the details of curves A and B respectively.

The main object of the work has been to determine the minimal amount of the various foodstuffs that must be added daily to the 'scurvy' diet in order to prevent occurrence of scurvy. When this minimal amount (or more) is consumed, growth does not cease about the twentieth day, but the animal continues to grow in good health without symptoms of scurvy (Curve F, Fig. 13). As a general rule the animals were kept under observation during three months.

The symptoms of severe scurvy in guinea-pigs are briefly the following: tenderness and swelling of the joints is the earliest sign, and the animal will frequently adopt a position (scurvy position), in which it rests on its side, while the painful member is held twitching in the air. In other cases the animal lies down with the side of its face upon the floor of the cage ('scurvy face-ache position'), indicating that its gums and jaw have become painful (see Fig. 14). Later on teeth become loose, solid food is refused, and death may occur a few days later. In cases where the anti-scorbutic given is only adequate for partial protection, the animal will usually live on for a considerable time, exhibiting swollen and painful joints, but growing up and enjoying a measure of health and spirits depending on the degree of protection afforded.

In case of death from scurvy the principal lesions at the post-mortem examination show close analogy with those characteristic of the human disease. Haemorrhages occur in any position, but are most frequent in the limbs. In cases where they occur in the intestinal tract blood is frequently passed in life, and death occurs suddenly. Rarification of the long bones is almost invariably present, and these frequently show fracture in the neighbourhood of the junction of shaft and epiphysis. The ribs are swollen and often fractured at the juncture of bone with cartilage.

Table VIII, which is drawn up by the staff working at the Lister Institute, shows the approximate anti-scorbutic value of a whole series of foodstuffs; the values given are based upon the minimal protective doses as determined for guinea-pigs. As in some instances the experiments are still incomplete, it is not possible to attach exact numerical values in every case. The series of signs are,

however, arranged to express the relative value of the various materials with as much exactness as is possible at the present time.

TABLE VIII. *Protective Value of Various Foodstuffs against Scurvy.*

0 = no value detected.

	Foodstuff.	*Value against Scurvy.*	*Min. Daily Ration to prevent Scurvy in Guinea-pigs.*
Cereals.	Whole grain	0	
	Germ	0	
	Bran	0	
	Endosperm, e.g. white wheaten flour, polished rice	0	
Pulses.	Whole dry	0	
	Germinated. (Lentils)	+ +	5 g.
Vegetables.	Cabbage, raw	+ + + +	1 g.
	,, cooked ½ hr. at 100° C.	+ +	5 g.
	Runner beans, green pods	+ + +	5 g.
	Carrot (juice)	+	20 c.c.
	Beetroot (juice)	+	More than 20 c.c.
	Swede (juice)	+ + +	2·5 c.c.
	Potato, cooked ½ hr. at 100° C.	+	20 g.
	Onion	+	
Desiccated Vegetables		+ to 0	60 g. expressed as equivalent in fresh cabbage.
Pickled Vegetables		0	
Fruits.	Lemon juice, fresh	+ + + +	1·5 c.c.
	,, preserved	+ +	5 c.c.
	Orange juice, fresh	+ + + +	1·5 c.c.
	Lime juice, fresh	+ +	10 c.c.
	,, ,, preserved	+ to 0	
	Grapes	Less than +	More than 20 g.
	Apples	,, +	
	,, dried	,, +	
	*Tamarind, dried	,, +	
	*Mango ,, ('Amchur')	,, +	
	*Kokum ,,	,, +	
Eggs.	Fresh, whites	?0	
	,, yolk	?0	
	Desiccated	?0	
Meat.	Raw (juice)	Less than +	More than 20 c.c.
	Tinned	0	
Milk.	Cow's, raw	Less than +	100 to 150 c.c.
	,, dried	Less than raw	
Yeast.	Pressed, autolysed	?0	
	Extract, 'marmite'	?0	

* Dried fruits much estimated as anti-scorbutic by natives of India.

Vegetables. Among the most potent anti-scorbutic materials are placed raw cabbage leaves, the raw juice of swedes, and the juices of the citrous fruits. Raw cabbage leaves take the first place, 1·5 grm. daily sufficing to protect from scurvy, or, in exceptional cases, as little as 0·5 grm. (Delf, 88). Among root vegetables there are great differences, raw carrot juice and raw beetroot juice proving comparatively feeble, while raw swede juice is placed in the front rank. The potato, which was tested in the cooked condition (after half-an-hour's boiling), would appear to occupy a mean position. In this connexion it is interesting to note that the cabbage and swede

are nearly allied species and belong to the same natural order of plants, viz. the Cruciferae. The old legend teaching that plants with a cruciform arrangement of the flower possess special virtue in the service of mankind thus receives scientific support at this late date.

Fresh fruit. Among the fresh fruits examined, the orange and lemon are easily the most valuable anti-scorbutic agents. It is therefore the more surprising to find that the juice of fresh limes is distinctly inferior, but such it has proved to be. The experiments on this point include tests with monkeys as well as guinea-pigs. Ordinary preserved lime juice was found to be almost devoid of anti-scorbutic properties, but preserved lemon juice appeared to be distinctly more satisfactory. Experiments are now in progress with the aim of discovering some method of preserving lemon juice which shall retain its original anti-scurvy value.

Dried vegetables and fruit. Dried vegetables, in confirmation of Holst's work, have been found practically useless (89, 90). From Delf and Skelton's results, cabbage leaves within two weeks of drying were found to have lost about 93 per cent. of their original anti-scorbutic value, while three months after drying less than 5 per cent. was retained.

The dried fruits examined were old specimens, probably six months old. Some degree of protection was detected in the small doses that could be administered.

Milk and meat. Milk and meat come last in the order of merit. Large quantities of fresh raw cow's milk, 100 c.c. daily and over, are required if scurvy is to be prevented by its agency alone (Chick, Hume and Skelton, 74, 75). In case of raw meat juice a daily ration of 20 c.c. offered only a very slight protection ; a larger ration was not tolerated.

Among the foodstuffs in which no definite anti-scurvy properties could be detected by the method employed are the following : eggs (both fresh and dried), cereals, malt (dried and kilned), preserved lime juice, autolysed yeast and yeast extract (marmite), pickled cabbage.

Resistance of the Anti-scurvy Factor to Heat, Drying, &c.

The absence of the anti-scorbutic principle from dried foodstuffs and its disappearance from powerful anti-scorbutics when these are reduced to the dry condition, is sufficient proof of the sensitiveness of the anti-scorbutic vitamine to drying. It may be regarded as an axiom that dry or dried foodstuffs will not prevent scurvy, and in this respect the contrast between the anti-beri-beri and anti-scurvy factors is very marked.

With regard to exposure to high temperatures, the anti-scorbutic factor is also much more sensitive than the anti-beri-beri factor, or the fat-soluble A factor. The experiments of Holst and Frölich upon this point have already been referred to, but more complete series of experiments upon this point have recently been carried out at the Lister Institute by Dr. Marion Delf (89). Working with

raw cabbage leaves the minimum daily ration required to prevent scurvy in guinea-pigs was determined to be less than 1·5 grm. and greater than 0·5 grm., i. e. about 1·0 grm. When the cabbage was heated in water at 60° C. for one hour, symptoms of severe scurvy were just prevented by a 5 grm. ration. When the temperature was 70°, 80°, 90°, or 100° C. for the same period, scurvy was not satisfactorily avoided. If the time was reduced to twenty minutes, the same ration, 5 grm., prevented scurvy when the temperature was 90° C. and just failed to do so when the temperature was 100° C.

The conclusion drawn from these results is that when cooked for one hour at temperatures ranging from 80° to 100° C., cabbage leaves lose about 90 per cent. of the anti-scorbutic value originally possessed (anti-scurvy value of 5 grm. ration reduced by cooking to the equivalent of about 0·5 grm. raw cabbage). In a similar manner the loss on heating in water either (*a*) for sixty minutes at 60° C., or (*b*) for twenty minutes at 90–100° C., was estimated at about 80 per cent. of the original (anti-scurvy value of 5 grm. reduced to the equivalent of about 1·0 grm. raw cabbage).

An interesting point emerging from these results is that the destructive influence of heat is enhanced to a comparatively slight degree with rise of temperature. Upon the above estimates, the rate of destruction is accelerated only about threefold (time required for destruction of 80 per cent. of the original value reduced to one-third) when the temperature is raised from 60° C. to boiling point, i. e. 40° C.

If the process of destruction by heat of the anti-scorbutic factor is assumed to be influenced by rise of temperature in an orderly manner, this result points to a temperature coefficient of about 1·5 for 10° C. rise of temperature. It is suggested by Delf that this low result may be of value in guiding speculation as to the character of the unknown anti-scorbutic factor, that it is opposed to the enzyme or protein-like theory of its nature and would argue a much simpler constitution. Destruction or denaturation of protein bodies by heat has been shown to possess a much higher temperature coefficient than the ordinary chemical reaction, which is accelerated 2 to 3-fold for 10° C. rise in temperature. The results obtained in a few typical instances are collected in Table IX.

At the same time it must be remembered that in Delf's experiment the anti-scorbutic material was contained in a tissue of vegetable cells, and it is possible that the low temperature coefficient may have reference to some extraneous limiting factor. Much further light may be expected from experiments now in progress on the effect upon anti-scorbutic value of heating the expressed juices of fruits and vegetables.

TABLE IX.

Substance.	Coefficient of heat change for 10°C. rise in temperature.	Observer.
Emulsin	7·14	Tamman (91)
Haemoglobin	13·8	Chick and Martin (92)
Egg albumin	635	„ „ (92)
Bacteria, disinfection by heat, *B. anthracis* spores	10	Ballner (93)
Bacteria, disinfection by heat, *B. typhosus*	136	Chick (94)
Vibriolysin	1,000	Famulener and Madsen (95)
Hydrolysis of cane sugar	3·6	Spohr (96)

The above experimental results confirm those of Holst and Frölich (70) in showing the great sensitiveness of the anti-scurvy factor to temperatures of 100°C. and below. They are also in marked contrast with the results obtained with the anti-beri-beri factor. In this case little significant destruction was detected on exposure to 100° C. for one hour or over (see above, p. 34). This greater instability of the anti-scurvy factor is a matter of the greatest importance in estimating the anti-scorbutic value of cooked vegetables and the relative merits of different methods of cooking, and will be referred to later under that heading, p. 64.

The anti-scorbutic factor is soluble in water and in alcohol (Harden and Zilva, 49; Hess and Unger, 97); it passes through dialysing parchment (Holst and Frölich, 70, p. 109), or a porcelain filter (Harden and Zilva, 49), without appreciable loss. The latter observers have also shown its behaviour towards adsorbents to be different from that of the anti-beri-beri factor; it is *not* adsorbed upon the surface of fine precipitates such as fuller's earth or colloidal iron. When a mixture of equal volumes of autolysed yeast and orange juice was treated with fuller's earth the anti-neuritic factor was removed while the anti-scorbutic remained unaltered.

Holst came to the conclusion that the anti-scurvy factor was more stable in acid than in neutral media. His opinion was based mainly upon the fact that acid fruit juices retained their anti-scorbutic properties much longer than vegetable juices. Recently Harden and Zilva (98) have shown that the presence of alkalis, even when dilute (1/50 normal sodium hydrate) and at room temperature, has a rapidly destructive effect upon the anti-scorbutic vitamine. These authors have called attention to the danger involved in the practice of adding sodium carbonate when boiling green vegetables.

It has been suggested that the loss in anti-scorbutic value suffered by vegetables during cooking might be lessened if the water in which they are heated were made slightly acid with citric acid. The suggestion was originally made in respect of germinated lentils in order to preserve the anti-scurvy value as far as possible where these may form the only source of anti-scorbutic material in a diet (99). It has, however, been shown experimentally by Delf (88) that, when 0·5 per cent. citric acid is added to the water in which germinated lentils are boiled, the loss in anti-scurvy properties is, if anything, greater than when no addition of acid is made.

It follows, therefore, that in cooking vegetables there should be no addition either of acid or alkali to the water in which they are boiled.

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CHAPTER IV

APPLICATION OF EXPERIMENTAL WORK TO THE PRACTICAL PROBLEMS OF HUMAN DIETS.

This section of the subject must necessarily be the one which arouses most interest, and it is, therefore, a matter for regret that our limited knowledge of the accessory substances should restrict to some extent the consideration of practical issues. Certain facts are, however, clear, and the application of these may be discussed with profit. The objection may be raised that the many theories concerning the accessory factors have been based solely on the results of experiments with animals such as rats, mice, guinea-pigs, pigeons, and, to a small degree, monkeys. There is, however, very little, if any, evidence that man's dependence on these factors is not as great as that exhibited by the rat or guinea-pig. We may assume that beri-beri and infantile beri-beri represent in man the ultimate result of a deficiency of the water-soluble factor, whilst symptoms of nutritive failure analogous to those shown by rats have been observed in young children suffering from a deficiency of the fat-soluble factor, and human scurvy is well known to result from a deficiency in the diet similar to that causing scurvy in guinea-pigs and monkeys. Evidence is also accumulating that accessory factors play a very important rôle in the growth processes of the higher animals, and many recent advances in animal husbandry have been based on these laboratory experiments with the lower species.

It is of the greatest importance to emphasize the fact that a deficiency of an accessory factor may be of a much smaller order than that necessary to produce the typical syndrome of the disease usually associated with that deficiency, but may nevertheless be sufficient to induce a distinct failure of nutrition and health. This is particularly true in the case of young children. Fortunately, in this country our natural dietary is so varied even amongst the poorer classes as practically to preclude an *absolute* deficiency of any particular factor arising, so that there is little fear of the incidence of a typical deficiency disease such as beri-beri. On the other hand, there is a very real danger that the improperly balanced dietaries consumed in many cases may lead to a partial deficiency of one or more of the accessory substances, if not of other components as well. The influence of these partial deficiencies, even when relatively slight, may be extremely serious when they occur in very early life, and, if we may judge from the experiments on animals, an adequate supply of these indispensable dietary components in later life may completely fail to make good the damage caused by the deficiencies in youth.

Table X shows the distribution of the three accessory factors in the commoner foodstuffs and will be found useful for reference in reading the following pages.

Table X.

Classes of foodstuff.	*Fat-soluble A factor.*	*Water-soluble B or anti-neuritic (anti-beri-beri) factor.*	*Anti-scorbutic factor*
Fats and oils.			
Butter	+ + +	0	
Cream	+ +	0	
Cod-liver oil	+ + +	0	
Mutton and beef fat or suet	+ +		
Lard	0		
Olive oil	0		
Cotton-seed oil	0		
Coco-nut oil	0		
Coco butter	0		
Linseed oil	0		
Fish oil, whale oil, herring oil, &c.	+ +		
Hardened fats, animal or veg. origin	0		
Margarine prepared from animal fat	Value in prop. to amount of animal fat contained		
Margarine from vegetable fats or lard	0		
Nut butters	+		
Meat, fish, &c.			
Lean meat (beef, mutton, &c.)	+	+	+
Liver	+ +	+ +	+
Kidneys	+ +	+	
Heart	+ +	+	
Brain	+	+ +	
Sweetbreads	+	+ +	
Fish, white	0	very slight, if any	
,, fat (salmon, herring, &c.)	+ +	,,	
,, roe	+	+ +	
Tinned meats	?	very slight	0
Milk, cheese, &c.			
Milk, cow's whole, raw	+ +	+	+
,, skim ,,	0	+	+
,, dried whole	less than + +	+	less than +
,, boiled ,,	Undetermined	+	,,
,, condensed, sweetened	+	+	,,
Cheese, whole milk	+		
,, skim	0		
Eggs.			
Fresh	+ +	+ + +	?0
Dried	+ +	+ + +	?0
Cereals, pulses, &c.			
Wheat, maize, rice, whole grain	+	+	0
,, ,, ,, germ	+ +	+ + +	0
,, ,, ,, bran	0	+ +	0
White wheaten flour, pure cornflour, polished rice, &c.	0	0	0
Custard powders, egg substitutes, prepared from cereal products	0	0	0
Linseed, millet	+ +	+ +	0
Dried peas, lentils, &c.		+ +	0
Peaflour (kilned)		0	0
Soy beans, haricot beans	+	+ +	0
Germinated pulses or cereals	+	+ +	+ +

Classes of foodstuff.	*Fat-soluble A factor.*	*Water-soluble B or anti-neuritic (anti-beri-beri) factor.*	*Anti-scorbutic factor.*
Vegetables and fruits.			
Cabbage, fresh raw	+ +	+	+ + +
„ „ cooked		+	+
„ dried	+	+	very slight
„ canned			„
Swede, raw expressed juice			+ + +
Lettuce	+ +	+	
Spinach (dried)	+ +	+	
Carrots, fresh raw	+	+	+
„ dried	very slight		
Beetroot, raw, expressed juice			less than +
Potatoes, raw	+	+	
„ cooked			+
Beans, fresh, scarlet runners, raw			+ +
Lemon juice, fresh			+ + +
„ preserved			+ +
Lime juice, fresh			+ +
„ preserved			very slight
Orange juice, fresh			+ + +
Raspberries			+ +
Apples			+
Bananas	+	+	very slight
Tomatoes (canned)			+ +
Nuts	+	+ +	
Miscellaneous.			
Yeast, dried	?	+ + +	
„ extract and autolysed	?	+ + +	0
Meat extract	0	0	0
Malt extract		+ in some specimens	
Beer		0	0
Honey		+	

DIET OF ADULTS.

Although there are reasons for believing that the adult has smaller requirements for the accessory substances than the growing child, the beneficial influences of a liberal supply cannot be denied.

There are some grounds for believing that the accessory food factors are concerned in the processes of tissue formation and repair, and we should therefore expect larger amounts to be necessary after exercise than after periods of rest. Whilst there is as yet practically no experimental evidence upon this point, there are indications based upon human experience that the demand for the three accessory factors is greater when work is being accomplished. This probability should be borne in mind when the dietaries or rations of those engaged in severe manual work, such as soldiers and labourers, are being considered (see p. 67).

For many years past the value of a generous dietary in combating diseases such as tuberculosis has been universally recognized, and it is a curious fact that many of the foodstuffs which enter so largely into the composition of these dietaries are those which are particularly rich in one or other of the accessory substances.

The Fat-soluble Accessory Factor in the Diet of Adults.

Considering in the first place the fat-soluble accessory it is noteworthy that milk, cream, butter, and cod-liver oil, all rich sources of this factor, form the basis of the treatment of diseases of malnutrition and of tuberculosis, and it must be admitted that such foodstuffs are more than mere sources of fat, otherwise the cheaper fats such as lard and the vegetable oils would long ago have been adopted as equally efficient for the purpose.

The very fact that the latter oils are not employed, or have been employed and rejected as of no value, suggests that some factor other than fat is operating in the case of milk-fat and cod-liver oil. In this there is an illustration of the results of scientific investigation justifying the selections based on long practical experience.

With regard to the fat-soluble A factor our present knowledge does not enable us to make definite statements as to the effect of a deficiency in the diet of an adult, but experience is much more advanced as concerns the water-soluble B (anti-neuritic) and anti-scorbutic factors.

The Water-soluble B (Anti-neuritic) Factor in the Diet of Adults.

Although the exact knowledge we possess regarding the distribution of the anti-neuritic accessory factors has been obtained by experiments upon birds, the analogy between avian polyneuritis and human beri-beri is so close that we may apply it without misgiving to the latter case. This conclusion is also justified by a careful study of the conditions under which human beri-beri has broken out and under which it has been cured. In many cases the records are incomplete, but their value is frequently enhanced by the fact that they were made by observers who were ignorant of the results of experimental work upon the subject, and when pieced together a mass of evidence is obtained which is in full accord with the principles set forth in the preceding chapter.

Beri-beri as the result of a diet consisting too exclusively of an over-milled cereal.

The classic example is found in the prevalence of beri-beri among the poorer classes in Japan, Malay States, &c., where rice is the staple diet. The researches of the investigators mentioned on p. 26, have succeeded in correlating the disease with the consumption of steam-milled, white, polished rice. Where the rice was merely husked and the outer skin and germ were retained, or where the rice was milled in domestic or native mills, so that the separation of these constituents was much less complete, beri-beri did not occur. Further, in localities where it is epidemic, beri-beri has been both cured and prevented by substituting whole rice or rice prepared in the native manner, for the fine, white, polished rice.

Eijkman (100) quotes an observation of Vorderman, which points forcibly to the connexion between beri-beri and highly milled rice. This worker made a survey of all the prisons in Java and Madura, noting the type of rice consumed and the incidence of beri-beri ; his observations extended over a total population of more

than 279,000 persons. The results are summarized in Table XI and show how closely the incidence of beri-beri varies with the degree of milling the rice has undergone.

TABLE XI.

Relations between the incidence of beri-beri in Javanese prisons and the type of rice consumed.

Type of rice consumed.	*Incidence of beri-beri.*			
	No. of Prisons examined.	*No. in which beri-beri occurred.*	%	*Proportion of cases of beri-beri among total No. of inmates.*
1. 'Half polished'; ¾ of the 'silver-skin' adherent	37	1	2·7	1 in 10,000
2. ½ of the 'silver-skin' adherent	13	6	46	1 in 416
3. 'Polished'; less than ¼ of the 'silver-skin' adherent	51	36	71	1 in 39

Apart from these general observations, there have been a few definite human experiments, which may be cited here. Strong and Crowell (101) obtained a number of volunteers from among the prisoners in a jail in the Philippine Islands, and these were placed upon a strictly controlled diet, divided into four groups. All received a certain amount of fish, bacon, lard, bananas, potatoes, and sugar, but rice was the staple article of diet and was provided in three different forms for the four groups of prisoners; the results were as follows:

Type of Rice.	*No. of men.*	*Cases of beri-beri.*
Group 1. White polished rice and extract of rice 'polishings' (bran)	8	2
Groups 2 and 4. White polished rice	17	13
Group 3. Red rice, i.e. whole, unmilled rice	7	1 (very slight)

This experiment suggests that when the diet consists mainly of cereal food, beri-beri can usually be prevented, if a whole, unmilled cereal is employed, but that there is not a great margin of safety. It will be noticed that the other subsidiary articles of diet were poor in content of anti-beri-beri vitamine.

Analogous cases of beri-beri outbreaks among wheat-eating populations are not so common. The peoples who are accustomed to consume white wheaten bread belong to the more highly civilized and richer countries, where the diet is varied and a sufficiency of anti-beri-beri factor is obtained from other constituents. There are, however, a few instructive instances among which the following four cases may be mentioned.

1. In Newfoundland and Labrador the population subsists largely on bread during the winter and spring. Formerly, when the bread was baked from 'brown' flour, beri-beri was unknown, as can be testified by the memory of the older inhabitants. At the present time, with the advance of civilization, the bread is made from pure white wheaten flour and beri-beri is frequent. Little (102) relates the following interesting occurrence. In 1910 a ship ran ashore, laden with a cargo of whole-meal wheaten flour, and in order to

lighten her a considerable portion of her load was removed and was subsequently consumed by the population in the adjacent districts. The result was that no case of beri-beri was reported in that region for a period of one year following this event.

2. Beri-beri was a rare disease on Norwegian ships before the year 1894. In that year an alteration was made in the sailor's diet, in response to a popular agitation to ameliorate the hard conditions of their life at sea. The sailors had previously lived on biscuit baked from rye flour (*in the milling of which there is no separation of germ*) ; after this date masters of ships were compelled to supply bread baked from white wheaten flour or a mixture of wheat and rye flour, and beri-beri became a frequent disease in the Norwegian mercantile marine (Holst, 103). There is an amusing story of one old sea captain who disapproved of the new-fangled reforms and insisted upon a supply of rye flour being taken on board for his own personal consumption. He was rewarded for his independence with the satisfaction of effecting cures among his men, who, when stricken with beri-beri, were supplied with biscuit from the captain's private supplies. As, however, these gradually showed signs of depletion, he was finally compelled to husband them in order to preserve his own health.

3. The deficiency in white wheaten bread is under ordinary conditions made good by the varied diet enjoyed by Europeans. If, however, the ' mixed diet ' is derived from tinned and preserved foods, the case is otherwise and beri-beri may be expected. This was no doubt the explanation of the beri-beri which has been reported among our troops in the Dardanelles and in Mesopotamia (Willcox, 104). In the latter campaign it is illuminating to note that the disease was confined to the British troops and was not reported among the Indian soldiers. This immunity was to be expected, for in addition to *atta*, a coarsely ground, whole-wheat flour, the native soldier receives a generous daily ration of *dhall* or dry pulses of various kinds, which are rich in anti-beri-beri vitamine. (See Tables II and III.)

4. The difference in the type of cereal ration issued respectively to the British and Indian troops was the basis of an unconscious ' experiment ', which took place during the siege of Kut. In his account of the medical arrangements during the siege of Kut-el-Amara (December 1915–April 1916), Colonel Hehir (105), I.M.S. writes :

' In the early stage of the siege, a recrudescence of beri-beri among British troops gave rise to some apprehension, but it then disappeared whilst in Indian troops and followers during the latter half of the siege, scurvy caused anxiety.'

At another place in this diary we learn that the British troops were receiving white wheaten flour until February 5, 1916, after which date they were compelled to take part of their flour ration in the form either of barley flour or of *atta*. It is very significant that beri-beri should have occurred while the British troops were enjoying white wheaten bread and should have cleared up when they were compelled to share the coarsely milled, germ-containing flour of their Indian comrades. The incidence of scurvy during this

siege showed an entirely opposite distribution; the British soldiers were protected by the large ration consumed of meat and horseflesh, while the Indian soldiers, largely vegetarian in habit, suffered terribly from this disease. (See also below, p. 64.)

The moral to be drawn from the above experience is that *for the prevention of beri-beri, it is important that the germ and bran of wheat should be included in the manufacture of bread or biscuit for any population living on a restricted diet. This is specially desirable in case of soldiers on active service where the rest of the ration may consist largely of tinned foods which may be regarded as vitamine-free, owing to the high temperatures at which they have been sterilized.*

In this connexion attention may also be drawn to two articles of diet, specially rich in anti-beri-beri factor and also specially suited to the need of armies on active service, viz. yeast extract and preserved eggs.

Yeast is one of the most valuable sources of the anti-neuritic factor and, in the form of an extract, gives the pleasant *savoury* taste to many of the soup cubes at present on the market. One commercial sample of such an extract (marmite) has been found on examination to retain the original value of the yeast from which it has been prepared, see Table II. The addition of such an item to the soldier's ration would be a useful and agreeable method of increasing the supply of anti-beri-beri vitamine in his diet.

Dried eggs have been found experimentally to retain the valuable anti-beri-beri properties of the fresh article. They probably are much too expensive an article to be included in the ordinary soldier's ration, but there can be no question of their suitability for inclusion in hospital stores. A large proportion of medical casualties on foreign service consists of intestinal diseases, and both during the acute stage and the subsequent convalescence the diet is largely restricted to bread, condensed or tinned milk, and invalid foods, which may be regarded as vitamine-free. It was noted by Willcox (104) that among twenty-six cases of beri-beri diagnosed in the autumn of 1915, eighteen had previously suffered from diseases of the digestive and alimentary system. It is not improbable that the restricted diet necessitated by these disorders may have been the predisposing cause of the beri-beri which occurred subsequently. The addition of eggs would have materially enriched these invalid diets in respect of anti-beri-beri properties, and in the form of dried eggs the addition could be made with the greatest convenience.

Before leaving this subject it may prove useful to give as an illustration the details of certain diets which have recently been found to occasion beri-beri, and to compare them with certain others, which under similar circumstances of climate, locality, &c., were found to be satisfactory.[1]

Diets A 1 and A 2, given in Table XII, refer to a camp in a Mediterranean area in which beri-beri occurred; they are essentially the same diet, but inasmuch as the rice ration was occasionally substituted by peas and beans, they form diets very different as regards the protection afforded against beri-beri. Both, however, are inferior

[1] For permission to publish the details of this investigation we are indebted to Major L. Braddon, R.A.M.C., and the Director-General of the Army Medical Service.

to Diets B and C, which were issued to similar neighbouring communities, in which no beri-beri was reported. After the diagnosis of beri-beri had been substantiated in case of certain men receiving Diet A 1/A 2, a careful search among the healthy men of the same community revealed a widespread abnormality in knee-jerks and other nervous reflexes. This is an important observation, as it shows definitely that normal health may be undermined by a deficiency in diet before any symptoms are apparent to the casual observer or to the individual himself.

TABLE XII. *Showing relative amounts of foodstuffs deficient and rich respectively in anti-neuritic factor, present in satisfactory diets and those found to occasion beri-beri; the amounts are expressed as oz. per week.*

Foodstuff.	*Nos. expressing approximate value in preventing beri-beri. Wheat germ = 100*	*Diet A (Beri-beri producing)* A 1.	A 2.	*Diet B (Satisfactory).*	*Diet C (Satisfactory).*
Foods deficient in anti-beri-beri factor:					
Rice		28		21	28
Bread (white flour)		224	224	112	168
Jam				7	7
Sugar		14	14	7	
Cheese		14	14	14	
Dry fruit				14	
Salt fish				20	
Margarine or butter or oil		14	14	7	3·5
X = Total No. of oz. weekly of foods deficient in anti-beri-beri factor		294	266	202	206
Foods richer in anti-beri-beri factor:					
Oatmeal	10			14	14
Fresh meat or bacon	10	42	42	30	42
Peas, beans, lentils	50		28	14	21
Potatoes (or fresh vegetables)	5	14	14	42	28
V = Total No. of oz. weekly of foods containing anti-beri-beri factor		56	84	100	105
Ratio V/X		0·2	0·3	0·5	0·5

In Table XII an arrangement is adopted by which foodstuffs deficient in the anti-beri-beri factor are collected in one group and those more valuable in this respect in the second. The points to be noticed are, firstly, the large preponderance of cereal food [1] in the diets A 1 (252 oz. weekly) and A 2 (224 oz.) compared with B (133 oz.) and C (196 oz.), and secondly the smaller proportion of vitamine-containing foodstuffs, A1, 56 oz. weekly; A2, 84 oz.; B, 100 oz.; C, 105 oz.

If the ratio—No. of oz. vitamine-containing food / No. of oz. vitamine-deficient food—be calculated for the four diets, we get

[1] Cooper and Braddon (106) found that the time and onset of polyneuritis in birds varied inversely with the quantity of polished rice fed to them; and that the amount of vitamine necessary for protection depended upon the size of the carbohydrate ration. Vedder (107), on the other hand, is of opinion that the size of the carbohydrate ration is without effect on the onset of beri-beri.

a series of values rising from 0·2 for diet A1 to 0·5 for diets B and C. It would appear as if this ratio might provide a useful indication of the value of a diet for the prevention of beri-beri.

The Anti-scorbutic Factor in the Diet of Adults.

In attempting to apply the results of experimental work upon scurvy in animals to the case of human diet, we are confronted with the same difficulty as in the parallel instance of beri-beri. The question whether guinea-pig scurvy and human scurvy are to be regarded as the same disease has, however, been answered in the affirmative, by those having experience of both diseases. In etiology, symptoms and methods of cure, the analogy between the two is so close that they may be regarded as pathological equivalents. In addition there are some scattered observations with monkeys in which the manifestations of the disease approach even nearer to those of human scurvy, especially as regards post-mortem appearances. These serve to bridge the differences existing between guinea-pig scurvy and the human disorder.

But valuable direct evidence upon this point is also found in the history of outbreaks of human scurvy, in cases where the details of the faulty diet and of the means successfully adopted for cure have been faithfully recorded. These instances may not offer convincing evidence when considered singly, but taken collectively they form an impressive confirmation of the experimental work and of the values assigned to the various foodstuffs in the second column of Table VIII. In point of fact a table of values constructed upon the sifted evidence of past human experience, would contain the foodstuffs ranged in an order not differing markedly from that constructed upon the results of experimental work. It is proposed to describe a few typical cases by way of illustration.

Fruit. The great value of fresh vegetables and fruit in the prevention and cure of human scurvy has so often been emphasized, that its reiteration has become a commonplace. The juice of fresh citrous fruits has for centuries been regarded as the anti-scorbutic material par-excellence. The ' experiment ' made by Dr. Lind (108) in 1747, quoted above, p. 39, is one of the most carefully recorded instances, but others are to be found in abundance.

Curran (109) mentions two ' hopeless ' cases of scurvy who received ½ oz. lemon juice with sugar and water thrice daily, and relates that the patients sat up and took food with cheerfulness in the course of two days. He also records that in the case of a gentleman affected with scurvy of a very severe description, ' the eating of a single rhubarb tart produced a most decided amelioration, equally sensible to the patient and his friends '.

Lind (108) recounts the tragic history of four ships which sailed from England to Bombay in April 1600, carrying 480 men on board, including merchants and other officials, in order to establish the East India Company. The Commodore upon his own ship had arranged for a regular issue of lemon juice, three tablespoonfuls daily, to all hands, and four months later, when the flotilla reached the Cape, his men were all in good health. On the other three ships,

however, the seamen were so severely attacked by scurvy that the passengers had to work as common seamen. In all 105 men died from scurvy during the voyage, and when Bombay was finally reached the entire work of unloading had to be performed by the crew of the Commodore's ship.

Budd (110) relates how the voyage of the Suffolk to Madras in 1794, which occupied nearly six months, during which no land was touched, was accomplished with almost entire freedom from scurvy. A regular ration of ⅔ oz. lemon juice was served out daily.[1] Scorbutic symptoms were noticed in a few men, but these disappeared on increasing the lemon-juice ration. Perhaps the most impressive instance of all is to be found in the history of the Navy itself, between the latter part of the eighteenth century, when thousands of cases of scurvy were reported annually, and the early years of the nineteenth century when, after the regular issue of lemon juice had been made compulsory in 1804, scurvy became a comparatively rare disease in the Navy.

Lime juice. The results of experimental work in Table VIII (p. 44) showing the inferiority of the juice of West Indian limes (*Citrus medica* var. *acida*), both in the fresh and preserved state, compared with that of lemons (*Citrus medica* var. *lemona*), are difficult to reconcile with the popular esteem in which the former is held at the present day as a preventive of scurvy. It is not, however, generally known that when 'lime juice' earned its laurels in the field of medicine, the term was used to signify the juice of lemons from the Mediterranean. Such was the case until the middle of the nineteenth century, when owing to favourable reports upon the acidity of the product, in which quality the anti-scorbutic virtue was held to exist, the supply of West Indian lime juice was gradually substituted. By the date 1870 very little, if any, lemon juice was issued officially.

This point has been investigated quite recently from a historical standpoint by Mrs. Henderson Smith (111), and the results of her inquiry afford an important confirmation of the experimental findings given above, p. 45. She has been unable to find any recorded instances in which scurvy has been prevented or cured by preserved lime juice, in absence of other anti-scorbutic agents. On the contrary, a careful comparison of certain Arctic expeditions in the fifties of last century, equipped with *lemon* juice, with those of later date provided with preserved *lime* juice has demonstrated the inferiority of the latter material. The most impressive case quoted is that of the Relief Expeditions sent in search of Sir John Franklin, 1847–59, where those ships which were supplied with lemon juice of good quality enjoyed remarkable immunity from scurvy, for long periods of time. One instance, that of the *Investigator* under Captain McClure, was specially noteworthy, for, in this case, there was no scurvy for twenty-seven months after leaving England, notwithstanding great privations. The event was otherwise in the *Alert* and the *Discovery*, two ships which left England in 1875 under Captain Nares, in an attempt to find the North Pole. These ships were equipped with all the improvements that the advance of

[1] In 1840 the navy ration was 1 oz. lemon juice daily with 1½ oz. sugar, served after two weeks at sea. (Budd, 110.)

twenty years had discovered and plentifully supplied with lime juice of the best quality. Notwithstanding these advantages, serious scurvy broke out at the end of the first winter spent in the Arctic regions. A commission of inquiry was held by the Admiralty on the return of these ships in 1876, but no satisfactory cause was found to explain the unexpected disaster. The commission took no notice of the fact that the 'lime juice' provided in 1875 was the juice of the West Indian limes, whereas in the fifties it had been the juice of Mediterranean lemons, but there is little doubt that in this difference lies the explanation of the different experience in the two cases.

Following this period a general distrust of 'lime juice' as a preventive of scurvy may be traced in the writings of those who had given careful attention to the history of later Arctic discovery, and the theory alluded to above, p. 38, that scurvy is due to chronic ptomaine poisoning, by tainted and salted meat, found ready acceptance. For example, the experiences of the Jackson-Harmsworth Expedition to Franz Josef Land in 1894–7 was accepted as being in accord with this theory (Jackson and Harley, 112) on the basis of the following facts.

The land sledging party was for three years without any lime juice and without any scurvy, but large quantities of fresh bear meat were consumed. The ship party, on the *Windward*, took their daily 1 oz. lime juice with regularity, and were well supplied with tinned and salted meat, but after one winter the whole crew developed scurvy and there were three deaths. There is no record that the meat was tainted or putrid when consumed, but it was afterwards assumed that it must have been so. In earlier days 'lime juice' had for so long been regarded as the equivalent in diet of fresh vegetables and fruit, that the supporters of the 'tainted' meat theory maintained further that fresh vegetables also were not concerned in the prevention or cure of scurvy, but that the disease was connected only with the quality of the meat eaten (see also below, p. 63).

Vegetables. Among the vegetables investigated the cabbage was found to be pre-eminent, and even after cooking the minimum preventive ration for guinea-pigs remained small in comparison with root and other vegetables. The following incident, related to Holst and Frölich (113) by a political refugee from Russia, bears testimony to the value of cabbage for the prevention of human scurvy, even when taken in the form of soup, after prolonged stewing. In a Russian prison, in which the narrator had been confined with 1,400 other prisoners, the diet consisted of tea, coarse bread, and cabbage soup. The preparation of the soup was so unclean that twenty of the inmates, including himself, who were of gentle birth and upbringing, could not endure to take it. After about six months these twenty prisoners showed symptoms of scurvy, while no case occurred among those who had consumed the soup regularly.

Among herbs and 'cresses' esteemed in the past for anti-scorbutic virtues it is interesting to note that those belonging to the natura order *Cruciferae* occupied a high place. This natural order includes also the cabbage and the swede, which were found to be the most valuable of the vegetables investigated experimentally. 'Scurvy grass' (*Cochlearia officinalis*), a small plant frequently found growing

near the sea shore, figures largely in old records of scurvy cures among mariners. Thus Bachstrom in 1734 tells the following story :

' A sailor in the Greenland ships was so over-run and disabled with scurvy, that his companions put him into a boat, and sent him on shore, leaving him there to perish without the least expectation of recovery. The poor wretch had quite lost the use of his limbs ; he could only crawl about the ground. This he found covered with a plant which he, continually grazing like a beast of the field, plucked up with his teeth. In a short time he was by this means perfectly recovered, and, upon his returning home, it was found to be the herb " scurvy grass ".' (Rendering given by Lind, 108, p. 395.)

Potatoes. Among roots and tubers the potato easily takes the first place in practical importance, not so much because of its intrinsic value, but because owing to its abundance, cheapness, and general acceptability large quantities are regularly consumed. It was not found possible to test it experimentally in the raw condition, but even when cooked, it was found to possess significant protective powers for guinea-pigs. There is no doubt that in northern climates the potato is of the utmost value in preventing scurvy during the winter and spring. Epidemics of scurvy have repeatedly followed failure of the potato harvest, e. g. in Norway in 1904, Ireland in 1847. The outbreaks of scurvy reported in Glasgow (114), Manchester (115), and Newcastle (Harlan, 116) in the spring of 1917 are doubtless to be attributed to the great scarcity of potatoes at that period.

Onions take a position between the more and the less potent vegetables. They possess a special importance, however, owing to the ease with which they can be transported, and are much appreciated whether raw or cooked by reason of their flavour. For these reasons they should always be included in rationing soldiers, sailors, or other communities of people at the end of long lines of communication cut off from fresh supplies.

Germinated pulses and cereals. There is so far little direct evidence of the anti-scorbutic value of germinated pulses in human diets. The custom of eating germinated cereals and pulses obtains in some parts of China (rice) and in the Malay States and Dutch Indies, where germinated beans, ' towgay ', are a common article of native diets ; there is, however, no evidence that the anti-scorbutic value of these foods has been recognized.

One instance, however, in which germinated beans were found to be of great value for the cure of scurvy is that recently recorded by Wiltshire (117), which yields complete confirmation of the experimental work described above. This observer succeeded in curing 27 cases of mild scurvy among Serbian soldiers (selected at random from a total of 57) by the sole measure of including in their daily dietary 4 oz. (dry wt.) germinated haricot beans. The progress was, if anything, better than that made by the remaining 30 patients who received 4 oz. fresh lemon juice daily instead. The beans were consumed after boiling for ten minutes only.

There is, however, indirect evidence to be gleaned in the high esteem with which certain fermented liquors made from germinated

seeds have been regarded for the prevention of scurvy. Captain Cook was a great believer in the anti-scorbutic virtues of a fresh infusion of malt (sweet-wort). His second voyage, 1772–5, was accomplished with an amazing record of good health among his crew, there being only four deaths during the period, of which one only was from sickness. He took a large quantity of 'malt, of which was made sweet-wort; to such of the men as showed the least symptoms of scurvy this was given, from one to two or three pints a day each man, or in such proportion as the surgeon found necessary. This was, without doubt, one of the best anti-scorbutic sea medicines then discovered, when used in time' (Captain Cook, 118, p. 227). According to Sir John Pringle, writing in 1776 (119), quas, 'a small brisk acidulous liquor', made from ground malt and rye meal, was regularly served out in Russian prisons as an anti-scorbutic.

Kaffir beer, 'leting' or 'joala', is another instance. It is the product of rapid fermentation of partly germinated millet and is consumed quickly after preparation. The Kaffirs are in the habit of taking large quantities when living in their own kraals in South Africa, and it is believed to be a valuable anti-scorbutic. It has been the custom of the mining companies on the Rand to arrange for the brewing of this beer in the compounds of the native labourers, and there large quantities are consumed. Outbreaks of scurvy have been reported by Dyke (120) among companies of Kaffir labourers in France, in cases where this 'joala' was replaced by a second type of beer, 'mahew', a fermented drink also made from millet and maize, but in the preparation of which *the grain is not previously germinated.* Modern beer appears, however, to be practically free from anti-scorbutic properties (Harden and Zilva, 121).

The use of germinated pulses as an article of diet certainly deserves a prolonged trial in circumstances where fresh food is scarce or unobtainable. Dry peas, beans or lentils contain less than 15 per cent. of moisture, are admirably adapted for transport, and can be germinated on the spot if and when required. Pulses of various sorts, as 'dhall', form a staple article of the native Indian diet, and had this knowledge of their value after germination been applied in Mesopotamia in 1915, it is possible that the terrible wastage from scurvy in that campaign (122, p. 72) might have been prevented. During the siege of Kut (December 1915 to April 1916) the British soldiers were protected from scurvy by large daily rations of fresh meat or horse flesh, but scurvy among the native soldiers presented a terrible complication. It is impossible to say whether the disaster of the final surrender might not have been averted if the 'dhall' ration for the Indian troops had been issued to them in the germinated condition.

Dried vegetables. Dried vegetables have repeatedly been tried and found useless for preventing human scurvy, and in this respect the results of the experimental work find abundant confirmation in the records of failure which have been preserved. Owing to the convenience with which they can be transported, dried herbs and vegetables have been repeatedly adopted for the supply of armies and other large bodies of men separated from supplies of fresh food; and with disastrous results.

As long ago as 1720, Kramer, chief surgeon with the Austrian army in Hungary, was confronted with a serious outbreak of scurvy among the troops and in his perplexity he wrote to Vienna for help and advice. The College of Physicians in that city arranged for a large and varied supply of anti-scorbutic herbs to be dispatched to his aid. The consignments arrived and were given a prolonged trial, but the result was that thousands perished from scurvy. Kramer epitomized the knowledge gained by his tragic experience in the oft-quoted paragraph in his *Medecina Castrensis*, 1720:

'The scurvy is the most loathsome disease in nature: for which no cure is to be found in your medicine chest, no, not in the best furnished apothecary's shop. Pharmacy gives no relief, surgery as little. Beware of bleeding: shun mercury as a poison: you may rub the gums, you may grease the rigid tendons in the knee, to little purpose. But if you can get green vegetables; if you can prepare a sufficient quantity of fresh, noble anti-scorbutic juices, if you have oranges, lemons, or citrons; or their pulp and juice preserved with whey in cask, so that you can make a lemonade, or rather give to the quantity of 3 or 4 oz. of their juice in whey, you will, without other assistance, cure this dreadful evil.' (Translation given by Lind, 108).

Dried vegetables were repeatedly tried in the navy, with similar results, and by the middle and end of the eighteenth century all persons who were well informed upon this subject were convinced of their uselessness for the prevention of scurvy. The following is a sample of what appears constantly in the correspondence of the Medical Board of the Admiralty at this period: *Digests of 'In' Letters*, 4806, 37. 1, August 22, 1773, Ref. M. Report from Commissioners for Sick and Hurt:

'On a reference of a letter from Earl Suffolk sending a box containing specimens of a plant and herbs prepared and dried for the purpose of curing the scurvy at sea. Acquaint Lord Suffolk they cannot recommend its introduction.'

And in 1757 Lind writes, in describing methods suggested for preventing scurvy at sea.

'The latest proposal to the Lords of the Admiralty was a magazine of dried spinach prepared in the manner of hay. This was to be moistened and boiled in their food. To which it was objected by a very ingenious physician (Dr. Cockburn), that no moisture whatever could replace the natural juices of the plant lost by evaporation, and, as he imagined, altered by a fermentation which they underwent in drying.' (Lind, 108, p. 143.)

When scurvy ceased to be a permanent menace this knowledge was soon forgotten, and Kramer's experience was repeated again in the American Civil War. In that campaign, large rations of dried vegetables, including dried potatoes, were issued to the men, but proved powerless to prevent scurvy, of which there were many outbreaks (123). At the present day it again needs to be emphasized that *vegetables which have been dried have lost almost the whole of their anti-scorbutic properties*. During the period of the present war the public have frequently been energetically advised in the daily press to dry and preserve vegetables for dispatch to prisoners of war

in Germany or to provide 'fresh' vegetables for our fleet when at sea (e. g. *Daily Chronicle*, October 19, 1917).

In case of *dried fruits* a distinct, though feeble, anti-scurvy value was detected in the experimental trials. This result also coincides with human experience. The dried tamarind, kokum, and mango ('amchur') mentioned in Table VIII were obtained direct from India, with the assistance of the authorities, and all possess a reputation in that country as anti-scorbutic materials. It is related that when scurvy broke out among our troops in 1833–4 at Nassirabad in Rajputana, great benefit was obtained by eating *anola*, the strongly acid dried fruits of *Phyllanthus emblica*, which is commonly sold in the bazaars (MacNab, 124). This difference between vegetables and acid fruits, in respect of the degree to which anti-scurvy properties are preserved in the dry condition, may be due to the influence of an acid medium in checking the process of destruction which the anti-scorbutic factor slowly undergoes after the cells of the plant have been disorganized by drying.

Meat. Many careful observers in the past have noted that the anti-scurvy value of fresh meat, though significant, was much inferior to that of vegetables or fruit. Curran (109) mentions three patients with scurvy at the Swift Hospital in Dublin in the epidemic of 1847, who had consumed ¾ lb. meat on five days in the week while developing the disease. Sir Gilbert Blayne relates an incident of the Fleet at Barbadoes in 1781, where a party of soldiers serving as marines were affected with scurvy and were sent to an army hospital where no fresh animal food was allowed. These men recovered much quicker on the vegetable diet provided than the seamen on the ships who were deprived of vegetables, but were fed on fresh meat.

There is no doubt, however, that scurvy can be prevented by the use of fresh meat alone if the ration is large. The history of Arctic experience is full of such cases. Dr. Rae, surgeon to the Hudson Bay Company, in his evidence to the Scurvy Commissioners of 1876, stated that among the inhabitants of that district scurvy was almost unknown. The people subsisted almost entirely on meat, but the amount consumed was upon the following scale, 8 lb. fresh venison daily per man, 4 lb. per woman, 2 lb. per child.

Nansen and Johansen, after leaving the *Fram*, spent two months, including the winter of 1895–6, on Frederick Jackson Island in a rudely constructed hut. They remained in good health and free from scurvy although obtaining no lime juice and no fresh vegetables and subsisting mainly on fresh walrus and bear meat preserved by cold.

Jackson and Harley (112) describe an interesting incident at Kharborova, Yugor Straits, where six Russian priests arrived in the autumn, attended by a small Russian boy. The priests by their religious vows were prevented from eating the fresh meat available: they subsisted on salt fish and there were no vegetables. In the following May the little boy was found to be the only surviving member of the party, and had buried all his late masters in the snow. He suffered from no religious disability and had fed largely on reindeer meat through the winter. A further instance is provided in the experience of Scott's first expedition (Voyage of the *Discovery*)

where an outbreak of scurvy was cured by the inclusion in the dietary of his party of large quantities of fresh seal meat. The case of the Jackson-Harmsworth Expedition referred to above, in the discussion of lime juice, is another instance of similar experience. The interpretation of these facts made by the leaders of the expeditions, was, however, to lead them to support the theory that scurvy was a disease due to chronic poisoning, by the ptomaine developing in tinned meat.

From Colonel Hehir's report on the medical history of the siege of Kut (105) it is clear that in this case also British soldiers were protected from scurvy by their regular ration of meat or horse flesh, but the amounts they consumed were considerable, 8 to 20 oz. daily. The Indian troops in Kut, on the other hand, who were vegetarian, suffered severely from scurvy.

Tinned and preserved meats can be dismissed in a word, as offering no possible protection from scurvy. Meat in its fresh condition contains the anti-scurvy factor in comparatively low concentration, and after exposure to the temperature necessary for sterilization it is impossible that any significant anti-scurvy properties should be retained.

The value of frozen meat is probably intermediate between that of fresh meat and tinned meat and, in any case, is likely to be low, especially if it has been for a long period in the frozen condition. Wiltshire (117), describing outbreaks of scurvy among Serbian soldiers, states that out of 132 cases in 1917 all had received a ration of frozen meat practically every day.

Influence of Method of Cooking upon the Anti-scorbutic Value of Cooked Food.

Seeing that the anti-scorbutic accessory factor is sensitive to high temperatures, it is clear that the value of fresh vegetables and fruit must of necessity be greatly impaired by cooking. When there is scarcity of fresh food, either by actual deficiency or by difficulty in transport or distribution, it is well to realize that raw fruit and salads have a value, weight for weight, far exceeding that of cooked fruit or cooked vegetables, and that a smaller ration will suffice to afford protection from scurvy. It is unfortunate that most fresh fruits and all salads are perishable articles of food and inconvenient for distribution. They are not available at all periods of the year nor are they universally acceptable. The fact remains that the bulk of the population in this country takes its anti-scorbutic food in the cooked condition, and this being so, the methods adopted for cooking become of great importance.

It is clear from the experimental results obtained by Delf (125) (p. 46 above) that over the range of temperature employed in ordinary cooking processes, variation in the temperature employed has a comparatively small influence upon the rate of destruction of the anti-scurvy vitamine. For example, if the temperature of cooking cabbage is lowered from 100° C. (boiling-point) to 80° C. (slow simmering) the rate of destruction of the anti-scorbutic factor is decreased only about two fold: in other words the loss in anti-

scurvy value caused by boiling for half an hour would be equalled by simmering for about one hour at 80° C. This time would, however, need to be greatly increased, with a consequent greater loss in anti-scurvy value, if the cabbage were to be rendered palatable. Cooking for a short period of time at the higher temperature is, therefore, much to be preferred to cooking for the much longer time necessary at the lower temperature.

For the preparation of food in large quantities methods of slow cooking are the more convenient, and hence arises the great popularity of the stew in camps and other situations where the arrangements for cooking are perforce of a temporary character. Methods of slow cooking at temperatures below boiling-point are also economical of fuel, and it is for this reason that the hay-box and other forms of 'self-cookers' are so widely recommended at the present time. When using these types of cooker the practice is to raise the saucepan containing the vegetables, &c., to boiling-point upon an ordinary stove, and after a short period of boiling (5–10 minutes) to place in the hay-box for two hours or longer. For one commercial hay-box it is recommended that cabbage should be left for 2½ hours after 10 minutes' preliminary boiling, and it is claimed that the temperature inside the saucepan will not fall below 97° C. If this perfection of non-conductivity be in reality attained, the net result as regards loss of anti-scorbutic value will not differ materially from the result of boiling for from 2–3 hours. The ordinary method of cooking cabbage by boiling for 20 minutes or half an hour is thus immensely superior in preserving the anti-scorbutic value. Some careful observations, upon an ordinary home-made hay-box (126), showed that cabbage was satisfactorily cooked with 5 minutes' boiling followed by 2 hours in the hay-box, during which time the temperature of the saucepan had, in fact, fallen to about 85° C. This operation may be assessed as equal to exposure to 100° C. for 5 minutes followed by exposure to a mean temperature of about 90° C. for 2 hours, or upon the basis of Delf's experiments, to about 1½ hours at 100° C., instead of the half-hour's exposure which would be the result of a simple boiling.

The conclusion to be drawn from these considerations is that all forms of slow cooking should be avoided, if possible, for those articles of food upon which dependence is placed for protection from scurvy. In case of fresh meat of inferior quality it is often necessary to resort to slow stewing in order to provide an appetizing meal, but in such cases, effort should always be made to cook the vegetables separately and for as short a time as possible.

In this connexion it is instructive to place on record two recent outbreaks of scurvy which have been attributed to neglect of this principle. Scurvy broke out in a camp in Scotland in the spring of 1917 and 82 men were affected. At the time potatoes were scarce, but the ration contained a fair proportion of fresh meat and 2 oz. of swedes were available daily. These, as will be seen from Table VIII, are among the most potent anti-scorbutic vegetables we possess, and, if cooked satisfactorily, should have afforded considerable protection. The cause of the outbreak was investigated by Prof. L. Hill, who discovered that the meat was always served as a stew,

the vegetables were added, and the whole cooked for about 5 hours. This circumstance was considered by Prof. Hill to be a sufficient explanation of the outbreak.

A second example is afforded by an outbreak of scurvy which broke out in a Kaffir labour battalion in France between May and July, 1918 (Dyke, 120), and in which 142 cases of pronounced scurvy were diagnosed. In this case there was a ration of fresh vegetables equal to 8 oz. daily, these were cooked with the meat and boiled for a period of at least 3 hours. In the opinion of the medical officer by whom the circumstances of the outbreak were thoroughly investigated this fact was an important contributory cause.

Period of Development of Beri-beri and Scurvy.

A diet deficient in both anti-scorbutic and anti-beri-beri accessory factors will occasion both diseases, but the likelihood is that symptoms of beri-beri will be the first to appear. In the 'ship berberi', described by Holst (127), the symptoms are reminiscent of both diseases, and it is probable that the cause is a deficiency in the diet of both factors.

The period of development of beri-beri has been determined with some care by Fraser and Stanton (128), who found the disease occurring among Japanese coolies after a period of eighty to ninety days upon a diet consisting mainly of polished rice.

In cases of scurvy the period of development is longer and probably not less than from four to eight months, as may be gathered from the following observations. Infantile scurvy is rarely seen in infants less than six months old, and according to Barlow (129) is most common about the eighth month of life. In the old days, when fresh food was scarce or absent during the winter, it was usually in the following spring, after several months of deprivation, that scurvy occurred. Colonel Hehir has stated that four months is the minimum time in which he has observed scurvy to develop among Indian troops on active service (122, p. 71). Holst and Frölich (113) quote two cases in which the period of development was observed to be longer. One is the case of a fanatical vegetarian in Christiania who wished to prove to the world that existence could be maintained on bread and water alone. During the period of his self-imposed trial he was guilty of backsliding on two occasions only, once for a pound of sugar and once for a bottle of beer. The bread was presumably rye bread and afforded adequate protection from beri-beri, but definite symptoms of scurvy in the form of haemorrhages in the legs were noticed after seven and a half months. This period of development is not very different from that in the narrative of the Russian refugee (see above, p. 59) in the Russian convict prison, where scurvy was developed after six months upon a similar diet.

There is a certain amount of evidence showing that these periods of development may be shortened in case of men performing hard manual work or exposed to damp or cold climatic conditions. In both cases metabolism would be stimulated, and it would be reasonable to suppose that any reserve of accessory factors would be the sooner exhausted (compare footnote, p. 56). The following are instances.

It was a matter of common observation that scurvy was specially prevalent among sailors exposed to cold, damp, or very rough weather. Lind (108) places this circumstance before diet in his dissertation on the 'Causes of the scurvy'. He gives instances of beleaguered cities where the garrison on hard duty succumbed to the malady before the inhabitants.

In the expedition of the *Alert* and the *Discovery* to seek the North Pole in 1875 the men wintered on the ships within the Arctic circle. There was no definite scurvy diagnosed during this period, although the diet was defective from the point of view of anti-scorbutic substances, notably by the substitution of lime juice for lemon juice. In the spring of 1876 the sledging parties set out, and with the performance of hard manual labour scurvy at once made its appearance, the first case occurring within ten days of departure. At first the officers escaped, but, as the men fell sick and the labour of dragging the sledges devolved more and more upon them, they also fell victims to the disease. In due course scurvy also broke out among the crews left behind upon the ships during the spring and summer of 1876, but its onset was distinctly later (Smith, 111).

A second instructive example is found in the outbreak of scurvy in the Scottish camp referred to on p. 65. In this instance the first cases to develop and a large majority of those showing severe symptoms were among a small section of the inmates who were engaged in hard manual work for a few hours daily.

One point of practical importance concerned with the slow development of these deficiency diseases is the certainty that long before the symptoms of the acute disease can be diagnosed there will be a general, ill-defined departure from good health which may defy diagnosis, but which will seriously lower the well-being and efficiency of the individual.

These considerations are especially important in cases where isolated cases of pronounced deficiency disease are diagnosed among large communities of people as in institutions, camps, &c. So small an incidence of the disease may be regarded as unimportant, but in reality it is an indication that a widespread danger exists and that the health of a much larger number of persons is being affected by the same cause, although, owing to greater natural resistance or other causes, severe symptoms may not for the moment have supervened.

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CHAPTER V

APPLICATION OF EXPERIMENTAL WORK TO PRACTICAL PROBLEMS OF HUMAN DIETS (*continued*).

ACCESSORY FACTORS IN THE NUTRITION OF INFANTS.[1]

PHYSIOLOGICAL evolution has elaborated in the milk of the mother a perfect food for the nutrition of the young during the period of infancy. The fundamental importance of the survival of the species has called into existence a wonderful provision for the maintenance of the composition of this foodstuff. Over and over again has it been proved (see Lusk, 131) that the mother will sacrifice her own tissues to provide the necessary components of her milk, should dietary restrictions call for such sacrifice. The normal milk of one species is naturally to be regarded as a perfect food for the nutrition and growth of the young of that species, and a certain relationship has been traced between the composition of the milk as determined by gross chemical analysis and the normal rate of development. This is only what is to be expected, for the requirements of a young animal for the tissue-building elements will be directly proportional to the growth impulse of the species to which it belongs.

But, as the recent work which we have surveyed has shown, no amount of protein and mineral matter will in themselves enable growth to take place. Nor will development be any more possible when these tissue-forming units are supplemented with an adequate supply of the energy-yielding foodstuffs. *Animals cannot grow without the accessory factors.*

Not only has that fact been established, but it is probably true that the rate of growth is closely related to the amount of these factors supplied, provided that the diet is adequate as regards its other constituents.

It must be remembered that, as in the case of other food components such as protein, an adequate supply of the accessory factors can only assist in giving the growth impulse full play. If larger amounts of these substances are administered than are necessary for normal growth, we do not get any supernormal development, for the upper limits of this are predetermined by the inborn capacity for growth.

What happens to the excess over and above that required for the normal development in such cases is not clear, but in the case of the fat-soluble A factor there are indications that like fat itself it is stored.

Since we have seen that these indispensable dietary factors appear to play a quantitative rôle in the processes of tissue formation, we

[1] See J. C. Drummond (130).

must admit the probability that the milk secreted by species with different growth impulses will show similar differences with regard to their content of these substances. Experimental evidence in support of the existence of such differences is as yet meagre, but it has recently been shown that cow's milk may be an inadequate source of the water-soluble B accessory for the growing rat (132), and of the anti-scorbutic for the growing guinea-pig (133).

The importance of differences of this type will be at once apparent when the problem of the artificial feeding of infants is considered.

Breast feeding. The fundamental importance of breast feeding cannot be over-emphasized, for it is now recognized as impossible to elaborate a perfect substitute for mother's milk. Much has been written to account for the superiority as food for the young of the milk secreted by the female of the same species. Such superiority cannot be ascribed to any one constituent. No small part of it is due to the fact that the mother supplies her young with a food which is both qualitatively and quantitatively adequate as regards protein, fat, carbohydrate, and inorganic salts. But no milk adequate only in these respects would be of any value for growth, and we must therefore ascribe the advantage of mother's milk for the nutrition of infants in part also to the presence of an appropriate supply of the accessory food substances unweakened by heat or dilution.

But it is most important to remember that it has been proved experimentally that the mother is entirely dependent upon her own food supply to provide her young with these substances, and should her diet be deficient in this respect they will suffer, sooner or later, in spite of any sacrifice she may make (134). We see therefore that the diet of pregnant or nursing women should be one rich in the accessory factors, so that they may be able to supply their offspring with a milk of high value in this respect.

Reference to the table of foodstuffs (p. 50) will indicate those which should be liberally supplied to expectant or lactating mothers.

In this country there is little fear of mother's milk being totally deficient in one or more accessory factors, but such cases are not unknown elsewhere; for example, it has been proved that infantile beri-beri, a disease frequently encountered amongst rice-eating populations such as the Philippinos, results from a deficiency of the water-soluble substance or anti-beri-beri factor in the mother's milk. Whilst, then, there is little chance of an insufficient supply of the anti-neuritic or anti-scorbutic principles to breast-fed children in Western Europe and America, there may be a very real danger of such children receiving an insufficient supply of the fat-soluble accessory. This will be the case where the mothers normally exist upon a dietary containing an inadequate amount of that factor, a condition which is probably far from infrequent among the poorer classes: for it must be borne in mind that the chief sources of the fat-soluble accessory—milk, butter, and eggs—are comparatively expensive foodstuffs. Studies of the dietaries consumed by the poorer classes have frequently been made; but although they have given results of value regarding the consumption of protein, fat,

and carbohydrate, they are of little use in determining whether the intake of accessory factors has been adequate.

In a recent study (135) it is stated that the fat content of the diets of the rural classes in this country is distinctly below that of the working classes in the cities. But it must be recognized that the fat consumed in rural districts is largely derived from dairy products, whereas the ever-increasing production of the cheap margarines lacking in the fat-soluble accessory may eventually tend to cause butter to disappear from the tables of the working people in the towns and cities. We cannot afford to neglect the fact that such a process may in time contribute towards lowering very considerably the nutritive value of the dietaries consumed by the poorer classes, in that there will be a tendency for an increased consumption of those cheaper foodstuffs which are in many cases seriously deficient in the fat-soluble factor.

Halliburton and Drummond (136) have experimentally shown that the cheaper forms of butter substitutes, i. e. the margarines prepared on a basis of vegetable oil or hydrogenated fats, are practically devoid of the fat-soluble accessory substance, and that only those margarines prepared exclusively from a fat such as beef-fat have any claim to possess a nutritive value approximating to that of dairy butter. Such facts as these cannot be disregarded in estimating the nutritive value of a dietary.

From a consideration of a number of the dietaries consumed by the poorer classes in the towns of this country, one is led to suggest that no inconsiderable proportion of the population is existing on a food supply more or less deficient in the fat-soluble factor. If this is so, it must follow that thousands of infants which are being nursed by the women of this population are indirectly being deprived of an adequate supply of that substance during the period of their life when it is vitally important that they should suffer from no dietary deficiency.

Artificial feeding of infants. A great deal of attention is being devoted at the present time to determining the most satisfactory method of rearing babies upon other foods than their mother's milk. Unfortunately this alternative to the natural method appears to be necessary in many cases, and it is therefore a problem of vital importance to ascertain the value of the many artificial substitutes for human milk.

In the past the aim has been to prepare food mixtures approximating in gross chemical composition to human milk in terms of protein, fat, carbohydrate, and inorganic salts. It is of the utmost importance that any modification made in milk intended for the feeding of infants should conform to the condition that an adequate supply of the three accessories should also be provided. Each of these requires consideration.

The Fat-soluble and Water-soluble (Anti-neuritic) Factors.

Cow's milk and modified milk. Some infants may be reared with success from a very early age upon unmodified cow's milk. The practical results which have been obtained by this method indicate

that ordinary untreated cow's milk is an adequate source of all three factors—fat-soluble A, water-soluble B, and anti-scorbutic—for the growth and nutrition of the human infant. This is in some respects what might be expected from the fact that the rate of growth of the calf is much greater than that of the human infant.

Other infants, however, are unable to take cow's milk until it has been modified so as to bring its composition nearer to that of human milk. Fortunately, the majority of formulae given for the preparation of what are erroneously termed 'humanized' milks recommend the use of milk products in the process of modification. This must now be regarded as a most important matter when considered in the light of our knowledge of the accessory substances, and it should be recommended that no product other than those derived from milk should be employed in the preparation of a modified milk used for infant feeding. Vegetable oils cannot replace cream, and sugar solutions cannot replace whey.

In some instances far too little regard has hitherto been paid to the character of the substances added to the milk in preparing infants' food. Thus, for example, a deficiency of fat has often been 'corrected' by the addition of various preparations containing olive oil, linseed oil, and cotton-seed oil. One such preparation which has been extensively utilized in this country is popularly known as Marylebone cream. This product is essentially an emulsion of linseed or olive oil. The following composition has been given (137):

Linseed oil	½ drachm.
Powdered gum acacia	8 grains
Essential oil of almonds	⅛ mn.
Elixir of saccharine (1 in 20) . . .	½ mn.
Water to make 1 drachm.	

This preparation is in many cases added to a dried separated milk, and Pritchard has stated (138) that the nutritive qualities of the linseed oil appear to be not inferior to those of cream. This is not in agreement with the carefully controlled experiments of McCollum in America (139), and already there appears to be some doubt in the minds of many pediatricians as to whether such emulsions are really efficient as substitutes for milk-fat. A modification of Marylebone cream is now prepared in which suet takes the place of linseed oil.

Apart from any question as to whether foreign fats of the type represented by linseed oil can replace the specific fat elaborated by the mammary gland, there cannot be the slightest justification for imperilling the health of a child during the most critical years of its life by denying it an adequate supply of the indispensable fat-soluble accessory substance.

Dried milks. We have already seen that fresh cow's milk appears to be adequate as a source of the accessory substances necessary for the growth of the infant, and the large number of young children which have been satisfactorily reared upon dried milk would indicate that the processes employed in desiccation have had no destructive effect upon the fat-soluble and water-soluble factors, although, as will be seen later, the anti-scorbutic principle suffers during the preparation.

The majority of dried milks are prepared by one or other of two processes, which may be termed respectively the ' drum ' and the ' spraying ' processes (137).

The ' drum ' process involves an exposure of the milk upon a revolving drum heated internally by steam at 140° C. The exposure is, however, a very short one, and is probably insufficient to cause any appreciable destruction of either of the two factors under consideration.

In the ' spraying ' process the milk is sprayed into a chamber through which a current of dry air at a temperature of 115° C. is passing. The dried powder falls to the bottom of this chamber and is, therefore, exposed to a temperature above 100° C. until it is removed

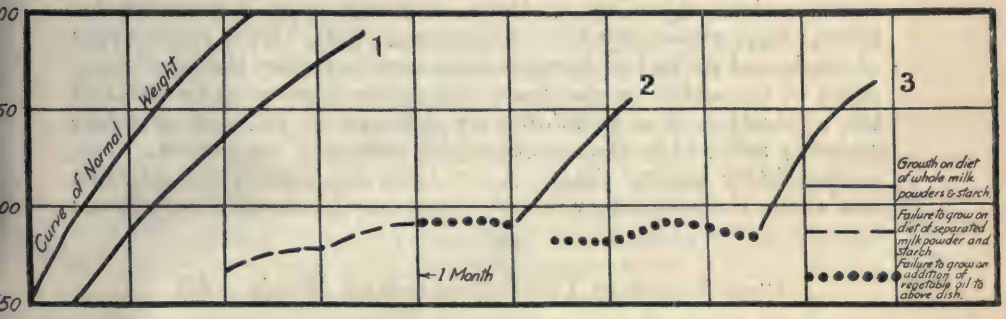

FIG. 15. These curves illustrate the inefficiency of vegetable oils such as linseed and olive oil to replace the fat of milk for promoting growth. Curve 1 represents the growth of a young rat fed upon a food paste composed of whole cream, dried milk, and starch. The growth curve is not quite up to the normal standard, but it is evident that there is no serious deficiency of either factor ' A ' or ' B '. The curves marked (— — —) show the failure of young rats to grow on a similar paste composed of dried skimmed milk powder and starch. This failure is due to the absence of an adequate amount of the fat-soluble accessory substance, and is not made good by the addition of linseed oil (curve 2 . . .) or olive oil (curve 3 . . .) to the ration in an amount equivalent to the deficiency of milk fat. Addition of butter fat, however, at once renders growth possible again (curves 2 and 3 ——).

for packing. It is probable that a certain amount of destruction of both factors might occur if the dried powder remained in the chamber longer than an hour.

It will be at once apparent that dried half-cream and separated milks will be deficient in the fat-soluble accessory. The curves given in Fig. 15 illustrate the failure of olive oil and lard to repair the deficiency of dried skimmed milk in the nutrition and growth of young rats.

' *Synthetic* ' *dried milk.* Coutts (137) has reported that a product prepared entirely from vegetable materials has been placed on the market as a substitute for dried milk. It is described as ' full cream synthetic milk ', and he has pointed out the undesirability of the use of the terms ' cream ' and ' milk '. It appears probable from what is known of the composition of this product that it would be entirely inadequate as a substitute for dried cow's milk for many reasons, particularly for lack of the fat-soluble vitamine.

Condensed milks. The usual process employed for the preparation

of the various types of sweetened condensed milks involves a preliminary 'pasteurization' at 80–90° C. followed by concentration at 50° C. under reduced pressure for two or three hours. It is doubtful whether such treatment would affect any serious destruction of the water-soluble accessory or the fat-soluble factor.

Condensed milks are classified according to whether they are full-cream milks or machine-skimmed milks. The former are often, and the latter almost invariably, sweetened by the addition of sucrose (cane sugar).

Any form of machine-skimmed milk, whether condensed or not, is unsuitable for the nutrition of the child, owing to the fact that its low content of fat, particularly after dilution, involves a correspondingly low content of the indispensable fat-soluble accessory.

The serious objections to the use of this type of foodstuff for infants have repeatedly been emphasized (140). With many types of condensed milks the dilutions necessary to reduce the sweetening effect of the added sucrose (cane sugar) are so great as to result in the production of a foodstuff very deficient in fat, and therefore seriously deficient in the accompanying accessory component.

Proprietary infants' foods. Apart from the foods prepared from cow's milk the proprietary infants' foods may be classified as follows:

Classification of Proprietary Infants' Foods (141).

(i) Foods with a basis of dried cow's milk but mixed with flour.[1]

(ii) Foods consisting mainly of flours, the starch of which is practically unaltered, or altered only by heating.

(iii) Foods consisting mainly of flours mixed with a proportion of malt flour or malt extract, but containing much unaltered starch which is not converted into soluble products during the process of preparing the food for infants in accordance with the directions given on the package.

(iv) Foods containing flours, but also containing active diastase or pancreatic ferment, so that if the food is carefully prepared according to the directions on the package the starch is appreciably altered.

(v) Foods manufactured from flours, the starch of which has been mainly or partially converted into soluble products during the course of manufacture.

As has already been pointed out by many authorities, the majority of proprietary infants' foods are seriously deficient in fat when prepared for use according to the directions given, and although we are not directly concerned here with the question of the influence of a fat-deficient or carbohydrate-rich diet upon infant nutrition, we cannot neglect certain aspects of that subject.

Many authorities have emphasized the dangers of a deficiency of fat in infant feeding, and many failures in nutrition have been ascribed to that cause. Hutchinson has stated that an abundance

[1] The word flour is used here to include not only the true cereals but other starch-containing vegetable substances, e.g. flour from leguminous seeds, arrowroot, banana, &c.

of fat should be the main characteristic of the diet in infancy, although care must be taken not to give it in an excess which would produce intestinal disturbance (142).

Numerous observers have reported a type of malnutrition in infants fed upon food mixtures poor in fat (143, 144). Such infants are frequently plump and well supplied with body-fat, and at first give the impression that they are well nourished, but they are usually found to be flabby, have a lowered resistance to disease, and are particularly prone to rickets. The question therefore arises as to whether the undoubted ill effects of a diet deficient in fat are actually a result of an insufficient supply of fat, or whether they may not be also due to a deficiency of the indispensable accessory substance associated with fat.

The majority of patent foods are seriously deficient in the fat-soluble factor, and it is probable that a number of them are also inadequate with respect to the water-soluble factor ; all are certainly deficient in the anti-scorbutic factor. Addition of milk to such foods will, of course, increase the amount of these factors present, but the resulting preparation will be inferior to milk in that respect. This fact constitutes the most powerful argument against the extensive use of these foods in infant feeding.

The symptoms of malnutrition that are observed in children as the result of prolonged feeding upon infant foods deficient in fat are curiously similar in many respects to those which may be observed in young animals deprived of an adequate supply of the fat-soluble accessory substance. Such cases show an unusually low resistance to bacterial invasion, although they may at the time possess a satisfactory body weight and appear plump and well. Cases have been observed in which the young born and nursed by female rats receiving a diet containing an inadequate amount of the fat-soluble factor have shown a normal rate of growth and, in many cases, may exhibit no obvious signs of malnutrition. But such offspring show, in the majority of cases, an unusually low resistance to infectious diseases, which only improves when they are given a richer supply of the accessory factor.

If this hypothesis regarding the cause of nutritive failure upon foods deficient in fat is correct, the adjustment of the fat content to normal limits by the addition of an oil or fat not containing the accessory factor, such, for example, as the vegetable oils, should effect little if any improvement in the health of the child, whilst the addition of an adequate fat such as cream, butter, or cod-liver oil should be followed by a rapid recovery.

Xerophthalmia in young children. It has already been described how young rats receiving an inadequate supply of the fat-soluble accessory substance not only show failure to grow but in most cases contract a characteristic disease of the external eye. This condition in rats fed upon inadequate dietaries was examined carefully by Knapp (145), who appeared to appreciate the relationship it bore to certain forms of conjunctivitis in children usually ascribed to faulty nutrition. He was, of course, unaware of the existence of the then undiscovered fat-soluble factor, but a study of the rations he employed shows that they were deficient in that respect.

Mori (146) has described a form of external eye disease prevalent in Japan, the symptoms of which bear close resemblance to those shown by the rats deprived of the fat-soluble factor. His work was also carried on prior to the discovery of this dietary component, but it is interesting to note that he approached very near the correct explanation when he attributed the condition to a deficiency of fat. He states that chicken livers, eel fat, cod-liver oil, and other fish oils are useful prophylactics, and reference to the list of foodstuffs (p. 22) will show that these are all of the type which are comparatively rich in the fat-soluble accessory.

More recently Bloch (147) and also Monrad (148) have described a form of external eye disease in young children in Denmark which they associate with a deficiency of fat.

Bloch gives a detailed account of forty-nine cases of this disease in babies treated at the Rijkshospital, Copenhagen, in 1912–16. All the patients had been artificially nourished, and the most severe and characteristic cases occurred in children about twelve months old, who had been fed on separated milk. The eye disease showed every stage of severity from xerosis of the conjunctiva in the slighter cases to hardening of the cornea, leading to ulceration, necrosis, and ultimate blindness in the more severe ones. Treatment of the eye condition in the ophthalmic department of the hospital gave little relief and no permanent improvement. On further study the children proved to be weak, anaemic, ill developed, apathetic, and with poor appetite. In some cases, especially of the younger children, the condition was associated with a condition of general atrophy. In the older children the general condition was not usually so severe, although the eye condition was frequently worse; there were often distinct swelling and oedema of the subcutaneous tissues, but no tenderness.

A careful scrutiny of the previous diets of the patients led Bloch to the conclusion that their general ill-health and the diseased condition of their eyes might be associated with a lack of fat in their food. Accordingly, while in hospital, they were given whole milk, cow's or human, and in addition as much cod-liver oil as could be tolerated. Under these circumstances a rapid recovery took place, both of the general weakness and of the specific eye disease. In absence of the cod-liver oil recovery was much delayed, and although it ultimately took place it was frequently too slow to save the sight.

In a later paper (149) Bloch describes a particularly interesting outbreak of eight cases of xerophthalmia taking place in a philanthropic institution for children in Copenhagen in the spring of 1917. The institution, which contained eighty-six inmates, was primarily intended for healthy children up to two years, but a number of ailing children were also included; these were housed in a separate building with the infants and in this department (section A) full milk was supplied; the children all did well and no case of xerophthalmia occurred. The second department of the institution (section B) contained the thirty-two older children, who were again divided into two subsections, B 1 and B 2, under different matrons. The eight cases of xerophthalmia all occurred in one subsection (B 1).

The diet, which was considered liberal and satisfactory, was the same throughout section B, with the exception that at breakfast

the children in B 1 had rusks and gruel and those in B 2 a beer and bread porridge with full milk. The midday meal consisted of soups or gruels made with milk, butter-milk, fruit syrups, barley, &c., &c., boiled fish, minced meat, mashed potatoes. The other meals consisted of cocoa, milk food, and various kinds of bread and margarine. The margarine was prepared from vegetable fats and the milk was all half-skimmed. No butter, eggs, or cream were used in this section of the home, and the only full milk was that taken with the breakfast porridge in the one subsection, B 2. This small amount, however, seems to explain the fact that all the eight cases of xerophthalmia occurred in subsection B 1.

These eight cases of xerosis of the conjunctiva were at first considered to be slight conjunctivitis, and local treatment with zinc salts was tried but with little result. It was then noticed that, whereas the growth and development of the children in section A had been satisfactory during the winter and spring, it had been subnormal in many instances in section B, in so far as many children had remained stationary or had lost weight during the recent months. This was true of the eight children who had developed xerophthalmia.

With the exception of two cases the eye condition was not severe. There is no mention of oedema, and the children, who otherwise appeared to be fairly well, though weak and without appetite, were allowed to be up and about. No change was made in the diet, except that 10 grm. cod-liver oil were given twice daily. In the course of a few days the xerophthalmia improved, and within eight days was cured. At the same time the children gained weight, and improved in general health. No other cases of xerophthalmia developed. The skimmed milk in the diet was afterwards partly replaced by full milk.

The author considers this disease to be one due to lack of the specific fat-soluble growth factor, and not to the lack of fat as such, which in the above instance had been supplied as margarine. The diet was, however, greatly deficient in the fat-soluble factor, owing to the use of skimmed milk, and cures resulted rapidly when cod-liver oil was administered.

In many of the other cases of xerophthalmia described by Bloch, and showing marked oedema, the children had been nourished on diets consisting too largely of carbohydrates and had been deprived of *all* fats. The author, therefore, makes the suggestion that lack of fat may itself be the cause of this and other oedemata arising from malnutrition.

Bloch considers this eye disease to be identical with that of Japanese children, called ' Hikan ', described by Mori (146), and successfully treated by the administration of fish- and other livers. The severer manifestations leading to blindness he considers due to superimposed infections. Throughout the work he calls attention to the frequency with which other diseases, such as diarrhoea, bronchitis, pneumonia, pyuria, discharges from ears and nose, and catarrhs of all sorts were found associated in these patients with xerophthalmia, showing a lowered resistance to infection resulting from their malnutrition. At the same time, it should be noted, especially in the light of the results discussed in a subsequent section of this report, that Bloch makes no mention of any signs of rickets

in most of the cases described. The children in many cases were not older than about twelve months, and it is possible that rickets may be a manifestation of a similar dietetic deficiency, less severe and extending over a longer period.

Anti-scorbutic Value of Milk and its Reference to Infant Feeding.

Experimental work has shown (Frölich, 150 ; Chick, Hume, and Skelton, 133) that if scurvy is to be prevented by the agency of milk alone, a quantity must be consumed which practically amounts to a complete milk diet. This result is in accord with the physiological rôle played in nature by mammalian milk, as a food specially adapted for the complete nourishment of the young during the early period of life.

Upon this basis fresh cow's milk must be classed among the less valuable foodstuffs in respect of anti-scorbutic qualities, and there is considerable evidence confirming this view. For example, Curran (151) mentions eighty cases among inmates of the Dublin unions in the Irish epidemic of 1847, who had consumed at least one pint of fresh milk a day for at least six months prior to the outbreak, although the diet had been deficient in fresh meat and vegetables.

There is a considerable amount of evidence that the diet of the mother has effect upon the vitamine content of her milk. It is possible therefore that human milk may be poorer in anti-scorbutic properties than cow's milk, although it must be admitted that there is no direct evidence upon the point. The breast-fed infant has the advantage, however, that its nourishment is taken in the raw condition. It is probable that infants brought up on cow's milk do not receive any great excess of anti-scurvy vitamine, and that this is further diminished when the milk is fed to the child after boiling or in the dried condition. Medical authorities are much divided upon this point, many maintaining that an infant will thrive upon boiled milk or dried milk, without the addition of any supplementary anti-scorbutic substance, others that such is desirable and even essential. For example, Barlow (152), in his ' Bradshaw lecture on Infantile Scurvy ', 1894, states his opinion that ' the boiling of cow's milk and prolonged sterilization (especially at high temperatures) lessens in some degree its anti-scorbutic quality '. Neumann (153) attributed the many cases of infantile scurvy, encountered in his private practice in 1901–2, to the practice of pasteurizing milk for infant feeding, recently introduced as a safeguard against transmission of bacterial infections. He found, on inquiry, that most of these infants had been receiving milk from one dairy in which it was the custom to ' pasteurize ' the milk at 90–95° C. before delivery, and that in their homes, the milk, as an extra precaution, had been heated a second time in a Soxhlet or other apparatus at or near 100° C. for ten to fifteen minutes. Heubner (154) has published similar experiences and, in common with Neumann, has expressed the opinion that the marked increase of infantile scurvy which he noticed in Berlin about the same period must be attributed to the fashion of pasteurizing milk before feeding to infants, a custom which had been recently

introduced and enthusiastically adopted even to the extent of repeating the operation more than once.

The best studied instance to be found in the literature is probably that recently recorded by Hess and Fish (155). These investigators have described an outbreak of very mild and sub-acute infantile scurvy in the Hebrew Infant Asylum, New York, among infants who had been fed for several months upon a diet of cow's milk previously heated to 63° C. (145° F.) for thirty minutes. It had always been the custom in this Institution to give orange juice as an extra anti-scorbutic to the babies fed upon pasteurized cow's milk, but it had been discontinued as a result of the pronouncement of the American Medical Milk Commission (1912) that for purposes of infant feeding heated milk might be considered the equivalent of raw milk. The result was that an outbreak of mild scurvy occurred two to four months later. The babies were not very ill, they were fretful, anaemic, had no appetite, and ceased to gain weight or grow. They were all over six months old, and it would have been reasonable to attribute the condition to teething troubles. The scorbutic nature of the illness was proved, however, by the ease and rapidity with which the symptoms cleared up when orange juice or other anti-scorbutic was restored to the diet, or when raw milk was substituted for the pasteurized milk. These conclusions of Hess and Fish have received confirmation in very similar observations made by Miller (156).

The whole question whether the nutritive value of boiled, is equal or inferior to that of raw, cow's milk is discussed fully by Lane Claypon (157), in whose book reference is made to many authorities who consider that there is no proof of any inferiority in case of heated or pasteurized milk.

It is not always stated, however, whether an extra anti-scorbutic was or was not employed with the diet of heated milk. Apart from this point it is probable that the difference of opinion existing on the whole question may be partly accounted for by differences in the length of time during which the milk was heated and the temperatures to which it was raised, and also by differences in susceptibility of different infants and in the periods for which they were maintained upon that diet without change.

As regards dried milk there is an exactly analogous difference of opinion among medical authorities. Pritchard (158) believes that ' when dried milk or other preserved foods are employed it is well to give some fresh fruit juice or other anti-scorbutic '. Naish, on the other hand (159), considers that ' the risk of scurvy upon such a diet is non-existent ', and adds that he is not in the ' habit of ordering any orange juice or any other anti-scorbutic '. His view is shared by Dr. Helen Campbell among others (see Coutts, 160, p. 90).

In the present state of our knowledge upon these points it is our duty to give our infants the best possible chance, and the wise course is to omit no precaution that may ultimately prove to have been necessary. An additional anti-scorbutic should therefore be given to infants who are reared on any artificial food, other than raw cow's milk. Even in this case, and that of the breast-fed infants, such a course might also prove beneficial.

As regards the most suitable anti-scorbutic material to be adopted, orange juice is easily the best from many points of view; it is a very potent anti-scorbutic, is convenient, needs no preparation, and is acceptable to the vast majority of infants. Under peace conditions oranges are cheap and plentiful, but during the war their scarcity and high price placed them out of reach of many poor mothers. The juice of raw swedes seems to be the best substitute for orange juice at the moment; the anti-scurvy value of these vegetables was found by Chick and Rhodes (161) to be very great and far in excess of the other root vegetables examined. Swedes have also the merit of being abundant and cheap. The preparation of the juice is exceedingly simple. The clean cut surface of the raw swede is grated on an ordinary kitchen grater and the pulp folded in a small piece of muslin and squeezed with the fingers, when the juice readily runs out. Its slight sweetish taste is not disliked by infants and it is already recommended in some infant welfare centres. Canned tomatoes have been found satisfactory by Hess and Unger (155 a). Where the presence of starch is not a drawback, cooked potato is also recommended by some workers (Barlow, 152; Hess and Fish, 155). The cooked potato is shaken up in water and the resulting fluid used as a diluent of cow's milk in the same manner as barley water.

For curative purposes potato cream, orange juice, or grape juice is usually employed. It has recently been shown, however (Harden, Zilva, and Still, 162), that lemon juice, from which the free citric acid has been removed by treatment with calcium carbonate and alcohol, is a valuable remedy inasmuch as, owing to the absence of acid, a very large amount can be administered without causing digestive disturbance.

A very serious effect of the anti-scorbutic deficiency in the guinea-pig is the degenerative change which occurs at an early stage in the odontoblastic cells of the teeth (Zilva and Wells, 162 a). It has not yet been ascertained to what degree a similar change occurs in infants or whether the permanent teeth are affected by an anti-scorbutic deficiency in early childhood.

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CHAPTER VI

RICKETS AS A DEFICIENCY DISEASE.

In consequence of the association of scurvy and rickets sometimes found in children, and further because of the efficacy of cod-liver oil as a therapeutic agent in rickets, it has naturally occurred to some (Hopkins, 163; Funk, 164) that this latter is also a deficiency disease, its development depending upon some dietetic error involving accessory food factors.

Experimental medicine has provided much positive knowledge concerning disease, but it has also shown how unreliable are many views on etiology when the basis is supposition and conjecture unconfirmed by scientific investigation. Reference to the literature of rickets (Findlay, 165) shows that it occupies a pre-eminent place among diseases whose etiology is surrounded by conjecture, and it is hardly an exaggeration to say that every specialist in children's diseases has his own hypothesis as to the causative factor of rickets. This portion of the monograph consists largely of a summary of an experimental investigation undertaken by E. Mellanby for the Medical Research Committee upon rickets in puppies (see Mellanby, 166). Although incomplete, the results are sufficiently definite to place rickets among the deficiency diseases.

This investigation carried out on puppies has consisted essentially in placing the animal on standard diets found to produce rickets, and then adding to the standard diets other substances in order to determine the effect of these on the development of the disease. As a result of the work it has been found possible to make a provisional arrangement of foodstuffs into those preventing and those not preventing rickets.

The standard diets used in the course of the work were as follows:

Diet I.	*Diet II.*	*Diet III.*	*Diet IV.*
Whole milk, 200 c.c.	Whole milk, 175 c.c.	Separated milk, 175 c.c.	Separated milk, (250–350 c.c.)
Porridge, Oatmeal	White bread	White bread (70 % wheaten)	White bread (70 %)
,, Rice	NaCl 1–2 gr.	Linseed oil, 10 c.c.	Linseed oil, 10 c.c.
2 gr. NaCl		Yeast, 10 grm.	Yeast, 5–10 grm.
		NaCl 1–2 gr.	Orange juice, 3 c.c.
			NaCl 1–2 gr.

The abstraction of the butter from milk and its substitution by linseed oil in Diets III and IV was carried out in consequence of results obtained with the earlier diets.

Similarly yeast and orange juice were added because they were found to improve the general condition of the animal and to allow better growth. In work of this nature it is obviously desirable to have such a diet as will allow the best possible general health and rate of growth consistent with a rapid development of the disease. As rickets is a disease that does not itself kill the child affected, and

FIG. 16. Rickets following a diet of 175 c.c. whole milk, white bread ad lib, and 10 c.c. linseed oil per diem. Time of experiment, $5\frac{1}{2}$ months. Increase in weight during period of experiment 2,670 grm.

[*To face p.* 83.]

in its development is most obvious in the well-grown child, it is evident that experimental work of this nature can only be satisfactory when good growth is obtained and the mortality is not abnormally high. It is true that puppies on Diets III and IV are very susceptible to extraneous diseases like distemper and mange, and these can only be excluded by taking great care of the animals. Under laboratory conditions a puppy taken from its mother at six weeks old and placed on Diet IV ought to develop well-marked rickets within six weeks.

The development and symptoms of rickets in puppies may be shortly described as follows (see Fig. 16). The bones are defectively calcified and the legs bend, the ligaments are loose and emphasize the leg deformity. Swellings at the epiphyses of bones are obvious, a rosary develops at the costo-chondral junctions, with other chest deformities according to the intensity of the affection. As in the rachitic child, the animals are lethargic and usually show great inability to run some time before the bone changes would prevent their so doing. That is to say, there is loss of tone of the muscle often accompanied by a lack of desire to carry out physical exercise.

After death the calcium content of the bones is low and the structure, as revealed by histological examination of the bones, is characteristic and pathognomonic, the irregularity of the epiphyseal cartilages and the presence of osteoid tissue in abundance being the most marked features. Skiagraphs of the wrists of two dogs of the same family, one on a rachitic and the other on an anti-rachitic diet, are shown in Fig. 17, A and B.

The following lists indicate roughly the effects of the various substances in preventing and allowing rickets when added to the standard diets (Table XIII). It will be necessary, however, to consider some of the substances more closely because their action occasionally varies under different circumstances.

TABLE XIII.

Substances not preventing Rickets in puppies.	*Substances preventing Rickets to varying extents in puppies.*
Bread ad lib.	Whole milk (500 c.c. a day).
Oatmeal.	Cod-liver oil.
Rice.	Butter.
Separated milk ad lib.	Suet.
Yeast, 10–20 gr. a day.	Olive oil.
Orange juice, 5 c.c. a day.	Arachis oil.
Linseed oil.	Lard.
Babassu oil.	Cotton-seed oil.
Hydrogenated fat.	Meat.
Calcium phosphate.	Meat extracts.
Sodium chloride.	Malt extract.
Meat protein.	
Milk protein.	

The substances having some preventive action on the development of rickets can obviously be classified into two categories—(1) the group of extractives and (2) the group of fats—and it is necessary to discuss these groups in turn.

Meat and malt extracts. In the earlier experiments on Diet II the addition of meat and meat extract definitely prevented rickets

(Diet II contained 5–7 grm. of butter-fat). Malt extract delayed the onset of the condition, and with large doses, e. g. about 20 c.c., some degree of protection was observed. When, however, Diets III and IV were the standard, the addition of meat (5 to 50 grm. in different experiments) did not prevent the development of characteristic symptoms. In all cases the meat seemed, however, to have an inhibitory influence which varied according to the experiment. For instance, meat added to the diet of a dog whose initial weight is small and whose rate of growth is also slight, may have a large influence on the development of rickets. With a large dog growing rapidly the influence of the meat is less evident, the increased metabolism in such a case apparently demanding a large supply of the anti-rachitic factor.[1]

The explanation of the different results obtained with meat, according as whether Diets II or IV were used, apparently depended on the fact that Diet II was not so powerfully rickets-producing because of the presence of 5–7 grm. of butter-fat in the whole milk. In itself the butter in the milk (175 c.c.) was not sufficient to prevent rickets, but its effectiveness was increased considerably by the addition of a small quantity (10 grm.) of meat per diem.

In addition to the above results, fresh vegetables (50 grm. potatoes and turnips per diem) have definite inhibitory action when added to the standard diets. This action is, however, not great, and is almost negligible in cooked turnip when the liquid is poured away after cooking.

Fats. It will be seen in the tables recording the rachitic and anti-rachitic substances that there is a wide variation in the effect of fats as regards rickets. In the first place, it can be definitely stated that the animal fats tested were more effective in preventing rickets than the vegetable fats, and the latter varied greatly among themselves. Linseed and babassu oils and a hydrogenated fat seemed to have no preventive action on the development of the disease. On the other hand, arachis and olive oils showed moderate preventive action, while oils like coco-nut and cotton-seed occupied an intermediate position. Such results demonstrate that the action of the oils does not depend on fats *per se* but rather on something these substances contain. If we call this something an anti-rachitic factor it is clear that cod-liver oil and butter contain much more anti-rachitic factor than linseed oil.

The Nature of the Anti-rachitic Factor.

The results obtained show clearly that diet plays an important part in rickets, and a study of the diets and experimental results seems to allow a satisfactory explanation along the lines of accessory food factors. It is generally agreed that there are at present three accessory food factors: (1) Fat-soluble A; (2) Water-soluble B; (3) Anti-scorbutic.

Two of these three factors can be immediately eliminated, as may be seen by reference to Diets III and IV. For instance, yeast is a rich source of the water-soluble B accessory, and since yeast in the diet

[1] See the analogous cases of beri-beri and scurvy (pp. 56, 67).

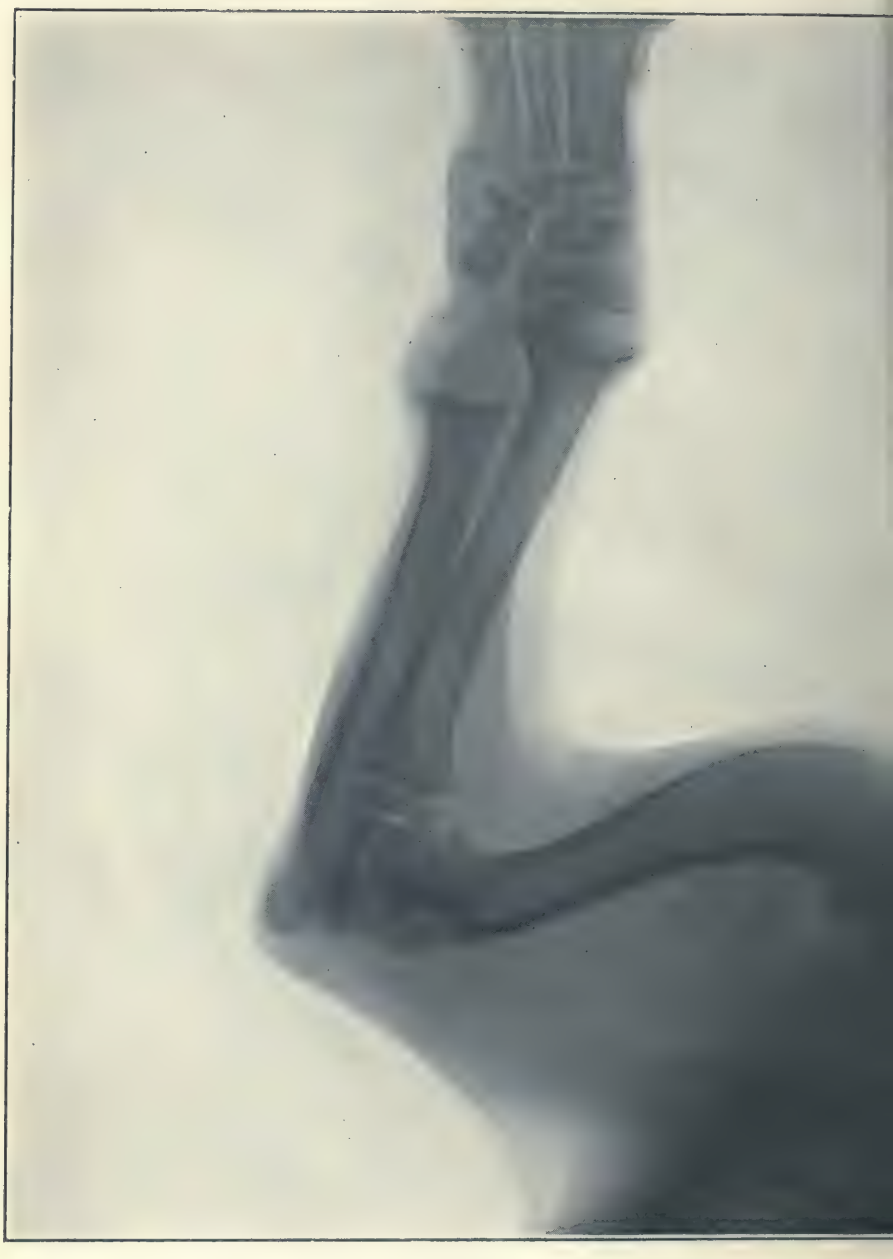
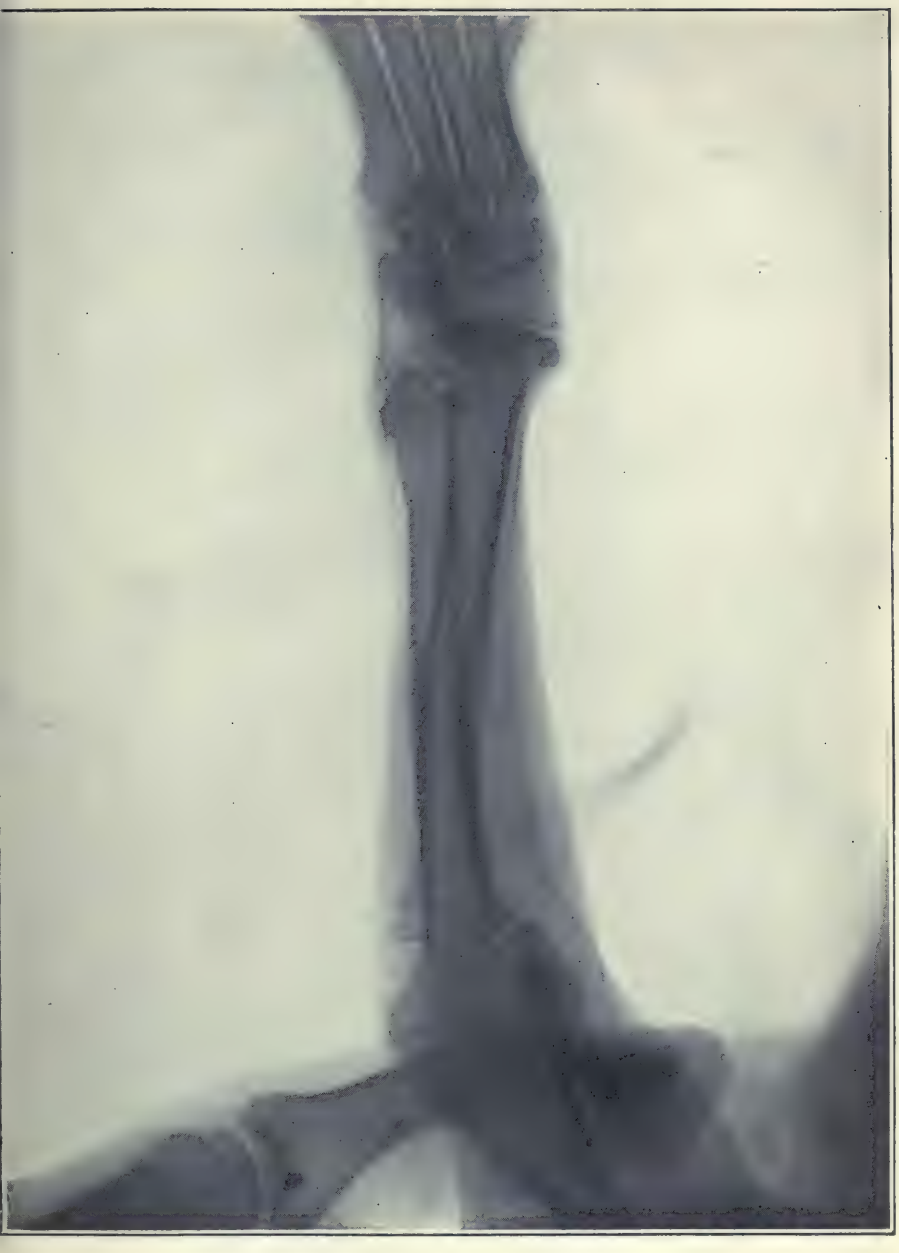


FIG. 17 A, B. SKIAGRAPHS OF

A. Duration of feeding, 3 months. Diet, 200 c.c. separated milk. White wheaten bread ad lib. Yeast, 5 grm. *Linseed oil*, 10 c.c. Orange juice, 3 c.c. Meat, 10 grm. Initial weight, 1,360 grm. Increase in weight in three months, 1,560 grm.

In these photographs B is normal outlines of the epiphyses and diaphy also the large amount of cartilage betw with B, this difference being more par results obtained in experiment of E.

[*To face p.* 84.]



TWO DOGS OF SAME FAMILY.

B. Duration of feeding, 3 months. Diet as A, except that 10 c.c. *cod-liver oil* was substituted for linseed oil. Initial weight, 1,845 grm. Increase in weight, 2,400 grm.

ll-developed rickets. Note the sharp
the bones in B as compared with A;
physes and diaphyses of A as compared
n in the case of the ulna of A. Above

is compatible with rickets and shows no inhibitory effect, it is certain that the water-soluble B accessory is not the anti-rachitic factor. Again, orange juice in the diet allows rickets to develop, and therefore the anti-scorbutic factor is probably not concerned. We are now left with the fat-soluble A factor, and although the identity of this with the supposed anti-rachitic factor is not completely proved, there is some probability that the substances are the same. It is known, for instance, that animal fats are a good source of the fat-soluble A factor and that vegetable fats are deficient. Similarly the anti-rachitic factor is more abundant in the animal fats than in the vegetable fats. On the other hand, as seen above, there is a well-marked grading of the anti-rachitic factor in the vegetable fats which was not traced in the distribution of the fat-soluble factor as studied in experiments with rats. It is possible that further work will show a more harmonious distribution of the fat-soluble A and anti-rachitic factors in the vegetable fats, for, in the main, the agreement is sufficiently striking to suggest that the differences may be a matter of technique.[1] Considering that the detection of fat-soluble A has depended on the feeding of young rats while the work on rickets has been carried out on puppies it is not surprising that such differences should appear.

It is much easier to prevent rickets in a small and slowly-growing puppy than in those of a larger type which grow rapidly. This is what might be expected from the accepted clinical observation that rickets attacks the large well-growing infant, and is not found in marasmic babies. In other words, rickets is a disease accompanying growth, and it appears that the more rapidly the animal grows the more anti-rachitic factor is necessary to keep the growth normal. Since, however, it is recognized that both fat-soluble A and water-soluble B are necessary for growth *per se*, in so far at least as rat feeding experiments are concerned, it might, at first sight, seem strange that a disease of growth should have its explanation in a deficiency of a growth-accessory factor. The experimental results indicate, that, however necessary it may be to have a minimum of fat-soluble A in the diet to ensure growth, it is by no means the case that the amount of growth has any relation to the amount of fat-soluble A eaten. On the other hand, there is an apparent relation between the rate of growth and the amount of anti-rachitic factor necessary to keep the growth normal and prevent the development of the rachitic syndrome. If, therefore, the anti-rachitic factor is the same as fat-soluble A, it appears from these experiments that the function of the latter is not so much to stimulate growth as to ensure the efficient working and normal development of organs and tissues.

Whether the undoubted anti-rachitic effect shown in some cases (on Diets I and II) by lean meat and meat extract is due to the comparatively small amount of the fat-soluble accessory contained in them, or whether the explanation of their effect must be sought along other lines, is a question requiring quantitative investigation.

It will be seen, therefore, that, although there is considerable

[1] Moreover, the influence of green leaves, a valuable source of the fat-soluble accessory, has not yet been examined in connexion with rickets.

evidence that fat-soluble A and the anti-rachitic factor are identical, further work is necessary, and the above difficulties must be cleared up before it can be stated definitely that they are the same.

Application of the foregoing Facts to other Hypotheses concerning the Etiology of Rickets.

The main hypotheses that have been put forward previously concerning rickets fall for the most part under two groups: (1) Dietetic; (2) Hygienic. There are, of course, other explanations, such as the action of the thymus (Matti, 167; Basch, 168; Klose and Vogt, 169) and other ductless glands, and the infection theory advocated by Koch (170), which cannot be so classified. It is obvious that an hypothesis involving ductless glands is not in antagonism to a dietetic explanation, more especially one concerned with accessory food factors, for nothing is known about the mode of action of the latter and very little of the former.

(1) Dietetic Hypotheses.

(*a*) *Rickets as a Disease due to Deficiency of Fat.*

The work of Bland Sutton (171) on the lion cubs at the Zoological Gardens has left its impression on English thought as regards rickets, and, together with the acknowledged efficacious results that follow the treatment of rachitic children with cod-liver oil and other fats, has brought about a general acceptance of the view that rickets is due to a deficiency of fat in the diet. The results here recorded make it clear that the efficacy of the treatment—curative and preventive—does not depend on the fat *per se*, but on whether it contains an abundance of anti-rachitic factor, animal fats being very superior in this respect to vegetable fats.

(*b*) *Excess of Carbohydrate in the Diet.*

When a diet contains excess of carbohydrate it means that it is made up largely of cereals. Now cereals, and more particularly cereals like wheat and rice, which have undergone some manufacturing process resulting in the loss of the embryo, are most deficient in accessory food factors. A diet, therefore, of such substances is unbalanced and most effective in producing rickets.

(*c*) *Deficiency of Fat and Excess of Carbohydrate* (Holt, 172; Cheadle, 173).

This condition comprises the first two hypotheses, and what is said about these can be extended to this suggestion. Such a combination would most certainly involve a deficiency of the anti-rachitic factor.

(*d*) *Deficiency of Calcium in the Diet.*

An abundance of calcium in the diet either in the form found in separated milk or as calcium phosphate will not prevent rickets when the diet is deficient in anti-rachitic factor. Similarly it has been found by Stoeltzner (174) and others that a diet otherwise

adequate, but containing too little calcium, will not produce rickets, although soft bones deficient in calcium salts are found in the animals. It is, however, probable that a deficient calcium intake associated with deficient anti-rachitic factor intake will bring about more strongly marked bone and other deformities associated with rickets than would occur if the supply of calcium salts were adequate. Lack of these salts must to this extent be an adjuvant factor in the etiology of rickets.

The above are some of the more commonly held dietetic explanations of rickets, and it will be seen that there is nothing in them which is contrary to the accessory food factor theory. In fact this suggestion in a general way focuses the above theories to a central point.

(2) Defective Hygienic Conditions.

The Von Hansemann (175) theory of domestication includes in a comprehensive way all the unhygienic conditions associated with life in civilized and especially in closely packed communities. The defects involved in civilized environment and more particularly met with in urban life are (*a*) dietetic, (*b*) involving confinement and lack of fresh air. The dietetic portion of this combination has been dealt with above and is considered to be the prime factor in the etiology of rickets.

The second factor—confinement and lack of exercise—will now be briefly discussed. The effect of confinement on the development of rickets has been studied by Findlay (176), who has decided that it is the real cause of the disease. Findlay's experiments involved the use of fourteen puppies, and during the experimental period of three months, five of these died. One on a diet of oatmeal and water and another on rice and water, died of marasmus. The remaining twelve were fed on porridge and milk (amount not stated), and of the nine animals that were confined, three died, one of marasmus, two of broncho-pneumonia with rickets. The confined animals were rachitic. Now, in an experimental research, designed to show the influence of confinement on the development of rickets, it is necessary that the diet used should be adequate and allow healthy development. That the diet given was not healthy is suggested by the high death rate recorded, and the experimental results only show that exercise is a factor in the production of rickets, but cannot be regarded as proof that it is the primary factor. Before the acceptance of this hypothesis is possible it must be shown that confinement on an adequate diet, that is to say, one compatible with the general health of the animal, always brings about rickets. In E. Mellanby's experiments puppies developed no signs of rickets during confinement for three months when fed on adequate diets.[1]

Reference may here be made to the recent statistical account of an investigation made by Miss Ferguson (178) on rickets, more particularly in Glasgow for the Medical Research Committee..

The interpretation of the results of this work are regarded as being adverse to the hypothesis that rickets is a dietetic deficiency disease,

[1] Findlay's work has recently been confirmed and extended by Paton, Findlay, and Watson (177).

and the general conclusion, although undetermined in a definite sense, is that the factors favouring the development of rickets are :

1. Insufficient space in houses.
2. Confinement in such houses.
3. Imperfect parental care.

No support is given to the diet hypothesis. Some of the dietetic results obtained by Miss Ferguson have been criticized elsewhere (166), and it remains doubtful whether accurate conclusions as to the diet of individual children, particularly of infants below two years of age, can be drawn from a consideration of family diet budgets.

Prophylactic Treatment of Rickets with Cod-liver Oil.

The therapeutic treatment of rickets with cod-liver oil and malt is generally regarded as efficacious, but the successful prophylactic trials made with cod-liver oil by Hess and Unger (179) gives additional weight to the view advanced in this monograph that rickets is a deficiency disease due to insufficient amount of anti-rachitic factor in the animals' diet. These workers arranged for the distribution of cod-liver oil among the children of negro families in the Columbus Hill District of New York. Rickets is rife in this district and is said to affect 90 per cent. of the whole number of infants. The oil was given to infants between four months and a year old, and the results obtained were so striking as to deserve reproduction here (Table XIV).

Table XIV.

Oil given. Average total.	*Duration of Therapy.*	*No. of Infants.*	*Infants not developing Rickets.*	*Infants developing Rickets.*	*Per cent. Non-Rachitic.*
54 oz.	6 months	32	30	2	93
23 oz.	6 months	5	4	1	80
21 oz.	4 months	12	7	5	58
Not given	—	16	1	15	6

It will be observed that, whereas without cod-liver oil 94 per cent. of the children developed rickets, when cod-liver oil was given in the largest dose, only 7 per cent. of the children were so affected. The cod-liver oil was a more potent factor than breast feeding, for even when breast fed almost all these coloured babies developed rickets.

The development of rickets in breast-fed babies is uncommon in England, but that it does occur is undeniable and demands the attention of research, for, in a normal way, an abundance of whole milk in the diet is incompatible with the development of rickets. Probably the explanation is to be found in the inability of the maternal organism to synthesize accessory food factors (see p. 70), so that a mother's milk is only adequate when she received a sufficiency of these substances in her own diet. It is interesting that Hess and Unger (180), as the result of a statistical investigation of the dietaries of negro women in New York, found a large proportion were eating too little fat ; also that the amount of milk they consumed was abnormally small, while vegetables and fruit only formed a small part of their diet.

General Considerations of Rickets as a Deficiency Disease.

It is but little realized how great and widespread is the part played by rickets in civilized communities. If the matter ended with bony deformities obvious to the eye it would be bad enough, but investigations have demonstrated that such deformities only represent a small part of the cases affected. Schmorl's (181) histological investigations on children dying before the age of 4 years showed that 90 per cent. had had rickets. The relation between defective teeth and rickets has been placed on an experimental basis recently by the work of May Mellanby (182), and there can be little doubt that any remedy which would exclude the one would almost certainly improve and might eradicate the other (see Fig. 18). The rachitic child carries the stigma of the disease throughout life in the form of defective teeth. Nor is this the most serious part of the evil, for the reduced resistance to other diseases of the rachitic child and animal is so marked that the causative factor of rickets may be the secret of immunity and non-immunity to many of the diseases which result in the high infantile death-rate associated with urban conditions. It is a striking fact to remember that in the West of Ireland, where the infantile death-rate is only 30 per 1,000, rickets is very rare, whereas in poor urban districts of this country where rickets is rife, the infantile death-rate varies from 100 to 300 per 1,000. It is at least suggestive that there may be some relation between rickets and the enormous death-rate of infants in towns even although the disease in itself does not kill. The experimental work with puppies has shown that the rachitic condition need not be at all advanced before the animal's whole behaviour is transformed. It becomes lethargic and is far more liable to be affected by such diseases as distemper, broncho-pneumonia, and mange. The low resistance of the animal to infection developed under conditions which ultimately may lead to rickets is impressive.

There is some danger in applying laboratory results to a clinical condition more especially when the results are new and for the most part uncontrolled by clinical observation. But some remarks are necessary in this connexion, for, if experimental research can point to the real cause of a disease, then not only is the curative treatment indicated, but, what is of much greater importance in the case of rickets, it ought to be possible to indicate why rickets is widespread and to direct action along preventive lines. It appears from this work that the foodstuffs of an infant ought to contain a maximum amount of anti-rachitic factor, and that the type and amount of fat eaten are important. Since the amount of fat a child can eat is limited, it is necessary to give children the best fat from the point of view under consideration. They should, therefore, not be given vegetable margarines or any other vegetable fat. The natural fat for a child is the fat of milk, and to give it a vegetable fat not only limits the amount of butter it can eat even if procurable, but also weighs down the diet in the rachitic direction. If additional fat is given, then cod-liver oil is the best.

Milk should remain the staple article of diet not only until weaning but for some years after this time. Milk is undoubtedly better than

the corresponding amount of butter. Under normal circumstances the child would then be assured of a good supply of anti-rachitic factor throughout its early life. Not, however, under all circumstances is this certain, for the work of McCollum, Simmonds, and Pitz (183) has shown that before an abundance of fat-soluble A appears in the milk the mother must have a good supply of this substance in her food. So far as the water-soluble accessory is concerned Drummond (184) has confirmed the results of these workers. This means that the animal's power of synthesizing these accessory food factors is small or absent. Green leaves are a good source of the fat-soluble A factor for the cow, and a well-fed cow, from this point of view, will give good milk. The nursing mother drinks this milk and eats eggs, butter, green vegetables, &c., and the accessory food factors are passed on to her mammary glands, thereby allowing the breast-fed child to get an adequate supply. If therefore a mother's diet is deficient in the anti-rachitic factor, it is easy to understand how even the breast-fed child may develop rickets. It is probable that the same argument applies even if it should subsequently prove that the anti-rachitic factor and fat-soluble A are not identical. These suggestions may also explain why in children brought up on cow's milk rickets develops more commonly in the winter months, when the cow's diet is more artificial and may contain less of the anti-rachitic factor.

As for the action of other foodstuffs it has been pointed out that meat has an anti-rachitic effect to some extent, and even in small quantities (10 grm. a day to a puppy) will render a slightly rachitic diet safe. Vegetable juices seem also to have some inhibitory action on the development of rickets.

Nowadays, when proprietary foodstuffs for children are much in evidence, the public should insist on knowing the relative value of all these preparations as regards their accessory food factors as well as their content of proteins, fats, carbohydrates, and salts. Synthetic milks, especially those containing vegetable oils, should not be given to infants and children unless they have been proved to contain an adequate amount of anti-rachitic factor. Similarly, dispensing vegetable oils to children instead of cod-liver oil ought to be discountenanced.

Finally, it may be stated that, although the experimental work here summarized deals only with young animals, it is certain that the necessity of having abundant anti-rachitic factor in the diet is also important at a later stage. Recently May Mellanby (182) has demonstrated the effect of diets deficient in an accessory food factor, probably fat-soluble A, on the calcification of puppies' teeth (see Fig. 18). Apparently the formation of calcified enamel and the adequate spacing of the teeth in puppies is dependent on an abundance of some such factor. In human beings calcification of the teeth is a much slower process and continues till the eighteenth year. In order to ensure perfect calcification of the teeth, therefore, it is necessary that the diet should contain adequate supplies of the accessory food factor up to this time, and a deficiency at any period will be reflected in a corresponding defect in the formation of the teeth. The teeth appear to be most susceptible to this deficiency and the

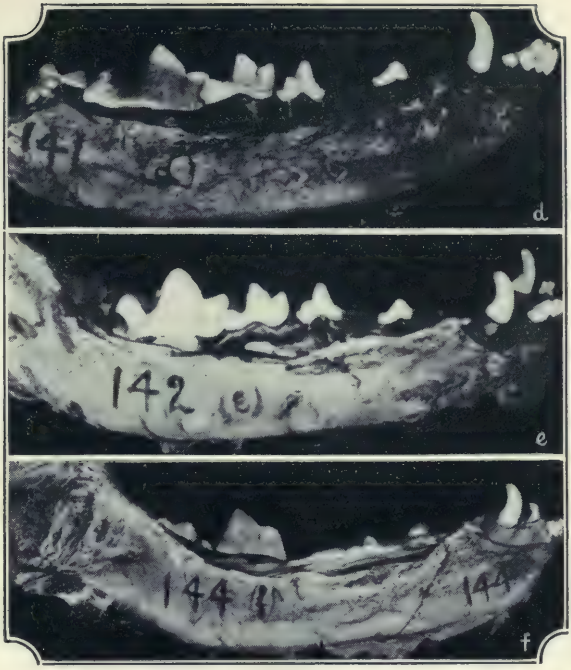


FIG. 18. Teeth of retriever puppies of same family. Age at death five months three weeks.

Diet. 175 c.c. separated milk ; white bread (70 per cent. wheaten) ad lib. ; linseed oil, 10 c.c. ; yeast, 10 grm.

In addition (*d*) received 5 grm. meat per diem.
(*e*) „ 10 „ „
(*f*) „ 50 „ „

From seventh to fifteenth week of experimental period (*e*) received 10 grm. of butter in addition.

Increase in weight during experimental period (15 weeks) (*d*) 3,330 grm.
(*e*) 4,145 grm.
(*f*) 5,135 grm

The enamel on most of the teeth of these dogs is poor, especially in the case of (*f*) ; it is best in (*e*) (butter). The size of the jaw in the case of (*f*) is in reality greater than is the case in (*d*) and (*e*). The magnification of the photograph is less.

The photographs show (1) the good effect of butter on the enamel (*e*),
(2) the fastest growing puppy (*f*) has the worst teeth.

(Reprinted from paper of May Mellanby (*Lancet*, December 7, 1918) by permission of the Editor of the *Lancet*.)

[*To face p.* 90.]

results suggest that the defective teeth of civilized man may depend on some such type of unbalanced diet as has been suggested as the cause of rickets.

In conclusion, there is good evidence that rickets is a deficiency disease due to diets which are unbalanced in that they contain too little of those substances rich in anti-rachitic factor and too much of those substances deficient in this respect. The anti-rachitic has, in many respects, a similar distribution to the fat-soluble A factor, and is possibly identical with this substance.

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CHAPTER VII

PELLAGRA.

Pellagra is a disease which is endemic in certain parts of the world, of which the chief are Northern Italy, Roumania, and the Southern States of North America. The disease may assume more than one form (Roberts, 185), but the characteristic symptoms in practically all cases consist of severe gastro-intestinal disturbances, accompanied by a bilateral symmetrical dermatitis which is painful and followed by desquamation and pigmentation. Until recently a survey of the literature on pellagra left the reader in obscurity regarding the etiology of the disease. The very multiplicity of theories that have been advanced was sufficient evidence that the correct explanation had not been discovered.

The majority of these theories may be divided into two groups, according as they regard the disease as one of *infective origin*, or as the result of a *faulty diet*. Of those investigators who lean towards the belief in an infective origin, prominence must be given to Sambon, who made a careful study of the disease in Italy (186). From an epidemiological study of the disease, he concluded that it was not connected with the consumption of maize, but that it was an infection, probably of a protozoal nature, and that flies of the genus *Simulium* (sand flies) were agents in the transmission.

Although this theory has received a certain amount of support (187, 188), it has failed to convince many investigators who could find no relationship between pellagra and the *Simulium* flies (189). In no case has the announcement of the isolation of a specific organism from cases of pellagra been substantiated by further and more careful investigation. The strongest evidence against the infectivity of the disease is, however, given by the failure which has followed all attempts to transmit it to animals and man (190 and 191). In spite of this, many still regard the disease as a specific infection, but no further progress has been made in proving the correctness of this view.

Turning now to those theories which ascribe the malady to dietary causes, it is found that to a large extent they originated in the observation that pellagra is frequently localized amongst people who subsist largely on a maize diet. Naturally the suspicion fell on maize, and for some little time considerable popularity was accorded to the theory which ascribed the malady to the consumption of diseased or spoilt maize (192 and 193). Several authors described the isolation of toxic substances from spoilt maize, and it was suggested that these products, produced by the growth of moulds, when ingested into the body were responsible for the production of a chronic intoxication resulting in pellagra. This and closely similar theories found many adherents, particularly in Italy.

The first suggestion that pellagra was a disease produced by a deficiency in the diet was made by Funk (194). Following the researches which he made upon the etiology of beri-beri, he attempted

to draw an analogy between that disease and pellagra. As has already been pointed out in an earlier section, the polishing of rice removes a certain substance, present in the embryo, which is indispensable for normal nutrition, and an absence of which from the diet eventually induces the train of symptoms associated with beri-beri. Funk suggested that in the milling of maize a similar substance was removed, and that an absence of that factor from the dietary would produce pellagra. He advocated the consumption of whole-meal maize and of potatoes to combat the disease (194). He was unable to produce the disease experimentally, and his theory, attractive as it might appear, was based purely upon conjecture. Since that time evidence has not been forthcoming which would justify the acceptance of his view, and a more careful study of the diet consumed in pellagrous districts is in itself sufficient to disprove the theory.

In 1914 Goldberger in America reported a very careful study of the dietaries of pellagrous communities, and his results clearly indicated a controlling influence of diet both in the causation and the prevention of the disease (195, 196, and 197). His work has led to the advancement of the most satisfactory theory yet put forward regarding the etiology of pellagra. The accuracy of his conclusions, drawn from the study just referred to, was demonstrated in a striking manner by his experimental production of the typical disease in man by feeding upon a restricted diet similar to that consumed in pellagra zones (198). The diet he employed consisted of dishes prepared from corn, patent flour, polished rice, pork fat, corn starch, syrup, sweet potatoes, cabbage and turnip greens. Vedder (199) has also expressed the belief that the disease may be the result of a deficient diet, and pointed out that it is localized in districts where the population subsist on low protein and high carbohydrate rations derived very largely from corn products.

In 1917 Chittenden and Underhill (200) reported the production in dogs of a pathological condition closely resembling human pellagra by restricting the animals to a diet of boiled peas, 'cracker meal', and salad oil (cotton-seed oil). By quantitative variation in the food intake, the condition could be produced in varying periods of time ranging from one to six or eight months. They found that the pathological condition usually began suddenly with a refusal to eat. After abstention from food for a day or two, the animals showed a characteristic condition of the mouth and tongue, which were covered with pustules. At the same time severe gastro-intestinal disturbances were indicated by a haemorrhagic diarrhoea. In many cases the condition could be cleared up and a normal nutrition re-established by the addition of meat to the diet, but the amount of meat had to be above a certain level, otherwise no improvement resulted. In fact, it was found possible to induce the pathological condition in animals fed upon a meat, cracker meal, and lard diet, when the intake of meat was kept sufficiently low. Chittenden and Underhill believe that the condition they have described, which they suggest may be analogous to pellagra in man, may be referred to a deficiency of some essential dietary constituent or constituents.

A systematic analysis of the deficiencies exhibited by the dietaries characteristic of those consumed in pellagrous districts has been made by McCollum (201).

As a result of the extensive researches which he has made on the nutritive value of various foodstuffs, it has been proved that the seeds of any plant (wheat, oats, maize, rice, beans, peas, flax, or millet), when fed as the sole source of nutriment, are inadequate for growth or prolonged well-being.

This has been traced to the fact that they all exhibit somewhat similar deficiencies. First, the quality of the proteins they contain is low. This is particularly true of maize, which contains considerable amounts of the protein zein, the molecule of which is deficient in the biologically important amino-acid tryptophan. Secondly, they are usually deficient in certain inorganic elements, particularly sodium, calcium, and chlorine; and thirdly, with the possible exception of flax and millet, they are inadequate sources of the indispensable food unit which is termed fat-soluble A (see p. 22).

McCollum has pointed out the most interesting fact, that the leaf of a plant may make good certain of the deficiencies of the seed. He has shown that cabbage and clover leaves are rich in the fat-soluble A and the inorganic elements sodium, calcium, and chlorine, and that a well-balanced food may be obtained by suitable mixtures of leaf and seed (202, 203). Working on this basis, McCollum has shown that the diets consumed by the populations of pellagrous districts show grave inadequacies, in that they are usually composed very largely of foodstuffs derived from seeds.

Thus, he has analysed the rations employed by Goldberger in the experimental production of pellagra in the human being (see p. 93), and has shown that 95 per cent. of the energy intake on those diets is derived from seed products or pork fat. Such a dietary shows three serious inadequacies:

1. It is undoubtedly deficient in the indispensable factor known as fat-soluble A.
2. It is probably deficient in sodium, calcium, and chlorine.
3. The quantity of proteins is low, and their nutritive value is below that of the proteins derived from meat or milk.

The following dietaries are typical of those consumed in the pellagrous districts of Italy (204), and represent the type of ration low in fat-soluble A and poor as regards protein supply:

I		II	
Maize	1,091 grm. daily.	Polenta	1,500 grm. daily.
Beans	60 ,,	Milk	100 ,,
Rice	67 ,,	Rice	100 ,,
Potatoes	67 ,,	Potatoes	100 ,,
Vegetables	250 ,,	Vegetables	100 ,,
Lard	21 ,,	Lard	20 ,,
Olive oil	33 ,,	Olive oil	10 ,,
Fish	67 ,,	Beans	100 ,,
Poultry	27 ,,	Cheese	50 ,,

The theory which regards pellagra as a result of prolonged maintenance on an ill-balanced dietary of this type has recently received additional support by the careful investigation recorded by Goldberger, Wheeler, and Sydenstricker (205).

A very exhaustive examination of the food purchased by pellagrous and non-pellagrous households in South Carolina was made. They showed that although the calorific intake of the pellagrous households was slightly less than that of the non-pellagrous, nevertheless the dietaries consumed by both types of household possessed calorific values comparing favourably with the recognized standards. The non-pellagrous undoubtedly enjoyed a larger supply of butter, and the animal protein foods, lean meat, milk, cheese, and eggs. Varying supplies of these foodstuffs, particularly fresh meat and milk, were associated with a corresponding inverse variation in the incidence of pellagra. In their concluding remarks they state: 'the indications afforded by this study would seem very clearly to suggest that the pellagra-producing dietary fault is the result of some one or, more probably, of a combination of two or more of the following factors: (1) a physiologically defective protein supply; (2) a low or inadequate supply of the fat-soluble vitamine; (3) a low or inadequate supply of the water-soluble vitamine; and (4) a defective mineral supply.'

'The pellagra-producing dietary fault may be corrected and the disease prevented by including in the diet an adequate supply of the animal protein foods, particularly milk, including butter and lean meat.'

An important series of observations have been made recently by W. H. Wilson (unpublished work) of Cairo, which throw new light upon the etiology of pellagra, and go to prove that the disease is to be attributed principally if not entirely to a defective protein supply in the diet. This conclusion emerged after a careful analysis of a large series of diets known to have produced pellagra, together with others proved to be preventive or curative. Special attention was paid to the protein provided, not only as regards the actual amount, but also as regards the 'biological value'. The figures expressing the latter are taken from the work of K. Thomas (206), who investigated the nutritive properties of protein derived from various foodstuffs, and estimated their relative value in maintaining nitrogenous equilibrium in the human subject. These values were found to vary within wide limits, the 'biological value' of vegetable protein (and especially of the protein derived from the maize grain) being less than that of milk or meat proteins. Equilibrium was not attained with maize diet, but Thomas calculated from the data obtained that the amount of 'tissue repair' effected by 30 grm. protein from meat or milk would require over 100 grm. of maize protein. Other foodstuffs, such as fish, rice, potatoes, occupied an intermediate position. The reason for this difference lies doubtless in the fact that animal proteins are more appropriately constituted for animal nutrition as regards the amount and variety of their constituent amino-acids. The inferiority of maize proteins would be explained by the large proportion contained of zein, a protein which is devoid both of tryptophan and lysine, two amino-acids which are known to be essential for animal nutrition.

Unfortunately the results obtained from the different experiments with maize were not concordant, and further confirmation is necessary before Thomas's figure for the biological value of maize protein can be accepted as final.

TABLE XV.

'Biological values' of various proteins, as measured by the relative 'tissue-repairing' values of equal weights.

Ox meat	104	Caseinogen	70
Cow's milk	100	Peas	56
Fish	95	Wheat flour	40
Rice	88	Maize meal	30
Potato	79		

It was after analysis of the pellagrous and non-pellagrous diets from this point of view that Wilson arrived at the conclusion that danger of pellagra occurred if the 'biological value' of the protein ration was below a certain level. This level, as might be expected, varied within certain limits according to individual need and idiosyncrasy, hard manual labour needing a more generous provision in this respect. This view is in accord with many of the known facts of the incidence of the disease. For example, upon this theory pellagra would be likely to develop with greatest frequency upon a diet consisting largely of maize, owing to the low 'biological value' of the proteins of this cereal; at the same time the disease might occur among wheat or rice eaters, in cases of an exceptionally low total consumption, but it could rarely occur among populations taking meat or milk even in small quantities. The protein deficiency which leads to pellagra and is possibly concerned with a defective supply of some essential amino-acid or acids may, in Wilson's opinion, occur also upon an adequate diet if assimilation is defective as the result of gastro-intestinal disease. This theory would account for certain rare cases, occurring in England and elsewhere, of a disease with symptoms closely resembling those of pellagra. In these instances there has frequently been a history of chronic diarrhoea and gastro intestinal disturbance of long standing.

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APPENDIX

[*The following memorandum was issued by the Committee on Accessory Food Factors in June* 1919*; it contains a short summary of the present state of our knowledge upon this subject and of the practical applications that can be made.*]

THE IMPORTANCE OF ACCESSORY FACTORS IN THE FOOD.

SOME FACTS CONCERNING NUTRITION, FOR THE GUIDANCE OF THOSE ENGAGED IN ADMINISTRATION OF FOOD RELIEF TO FAMINE-STRICKEN DISTRICTS.

Recent research has shown that the requirements of the human organism as regards diet cannot be met entirely by an adequate supply of protein, fat, carbohydrate, inorganic salts, and water. It has therefore modified the common belief of ten or more years ago, when the attention of physiologists was focussed upon the calorie or energy value of the diet. It is now established that, in addition to these necessary constituents, certain unidentified principles, known as accessory food factors or ' vitamines ', must also be present in order to maintain health and prevent the occurrence of ' deficiency diseases '. These substances have not so far been isolated, little is known of their chemical or physical properties, and at the present time their presence can only be detected by experiments with animals.

These accessory factors or vitamines are widely distributed among naturally occurring foodstuffs, and in time of peace, under normal conditions of food supply, the variety of food consumed by European nations protects them from risk of any deficiency in these essential substances. Under the conditions arising from the war a different state of things exists ; in addition to a general shortage of food there is also a great restriction in the variety available, and danger from ' deficiency diseases ' is to be feared.

Of these diseases scurvy is the best known, and the belief that it is caused by some deficiency in the diet has long been strongly held. Recent research has added to the deficiency diseases beri-beri, rickets, and other less well-marked disorders of growth and departures from health.

The following notes have been compiled by the Committee on Accessory Food Factors in the hope that they may afford practical help to those occupied in the administration of food relief to the famine districts of Eastern Europe. The advice given is based upon the present state of our knowledge of the distribution of accessory food factors (vitamines) in natural foodstuffs and of the rôle played by them in preventing disease and in promoting health and growth.

The accessory food factors at present recognized are three in number :

(1) Anti-neuritic or anti-beri-beri factor, identified with the water-soluble B growth factor of the American investigators.

(2) Fat-soluble A growth factor or anti-rachitic factor.
(3) Anti-scorbutic factor.

As far as is known the accessory food factors cannot be produced by the animal organism, and all animals are dependent for their supply directly or indirectly upon the plant kingdom.

Distribution and Properties of the Accessory Factors.

(1) **Anti-neuritic or Anti-beri-beri Factor** ('water-soluble B' growth factor of the Americans).

This vitamine prevents the occurrence of beri-beri in man and analogous diseases in animals. It is also necessary to promote satisfactory growth in young animals. It is widespread, and is found to some extent in almost all natural foodstuffs. *Its principal sources are the seeds of plants and the eggs of animals,* where it is deposited, apparently, as a reserve for the nutrition of the young offspring. Highly cellular organs such as the liver and the brain contain considerable amounts of this vitamine; flesh contains comparatively little. Yeast cells are a rich source, so also are yeast extracts, e.g. 'marmite'. In the case of peas, beans, and other pulses, this vitamine is distributed throughout the seed, but with cereals it is concentrated in the germ (embryo) and in the peripheral layer of the seed which in milling is peeled off with the pericarp and forms the bran.

Beri-beri is occasioned by a diet composed too exclusively of cereals from which germ and bran have been removed by milling, as in the case of polished rice or white wheat flour. The disease is common where polished rice is the staple article of diet to the almost entire exclusion of other foodstuffs. It is rare, though not unknown, where white wheat bread is eaten, because the consumption of this type of cereal food is usually accompanied by a sufficiency of other foodstuffs containing the essential principle. It is unknown where rye bread is the staple food, because in the milling of rye there is no separation of the germ.

(2) The Fat-Soluble A Growth Factor or Anti-rachitic Factor, necessary to promote Growth and prevent Rickets in young Animals.

This vitamine appears to be necessary also to maintain health in adults, and it has been suggested that war oedema may be due to a lack of this factor in the diet.

The main sources of this factor are two in number:

(1) certain fats of animal origin,
(2) green leaves.

The most notable deposits of this factor are in cream, butter, beef fat, fish oils (for example, cod-liver oil, whale oil), egg yolk. It is present in very small or negligible amount in lard (pig fat) and in vegetable oils, as, for example, linseed oil, olive oil, cotton-seed oil, coco-nut oil, palm oil; pea-nut or arachis oil is reported to contain it in larger amount. It will be noticed that this factor is found chiefly in the more expensive fats.

While green-leaf vegetables contain the fat-soluble factor, root

vegetables are deficient in it; war oedema has been frequently reported under circumstances in which root vegetables have formed a large proportion of the diet.

(3) **Anti-scorbutic Factor.** This vitamine is necessary in a diet for the prevention of scurvy, and is found in fresh vegetable tissues and (to a much less extent) in fresh animal tissues. Its richest sources are such vegetables as cabbage, swedes, turnips, lettuce, watercress, and such fruits as lemons, oranges, raspberries, tomatoes. Inferior in value are potatoes, carrots, French beans, scarlet runners, beetroot, mangolds, and also (contrary to popular belief) lime juice. Potatoes, although classed among the less valuable vegetables as regards anti-scorbutic value, are probably responsible for the prevention of scurvy in northern countries during the winter, owing to the large quantities which are regularly consumed.

Milk and meat possess a definite but low anti-scorbutic value.

This vitamine suffers destruction when the fresh foodstuffs containing it are subjected to heat, drying, or other methods of preservation.

All dry foodstuffs are deficient in anti-scorbutic properties; such are cereals, pulses, dried vegetables, and dried milk.

Tinned vegetables and tinned meat are also deficient in anti-scorbutic principle. In case of tinned fruits the acidity of the fruit increases the stability of the vitamine, and prevents, to some extent, the destruction which would otherwise occur during the sterilization by heat and the subsequent storage.

A table giving a summary of our knowledge as to the distribution of these three accessory factors among the commoner foodstuffs is appended, p. 102.

Practical Application of the Foregoing Facts to the Prevention of Disease.

(1) Prevention of Beri-beri.

It is unlikely that any danger of beri-beri will arise among the famine threatened districts of Eastern Europe *as long as wholemeal flour from rye, wheat, barley, maize, or peas, beans, and lentils are provided.* Mere shortage of food does not cause beri-beri, and poverty ensures that the whole grain is consumed for purposes of economy.

(2) Prevention and Cure of Rickets or Growth Failure in Children or War Oedema in Adults.

Evidence is accumulating that rickets is caused by a shortage not of fat as such, but of the 'fat-soluble growth factor' which is contained in certain fats. Xerophthalmia, a severe disease of the external eye, leading, if untreated, to blindness, has also been attributed to lack of this factor. Infants and young children must therefore be supplied with the *right kind of fat.* To prevent rickets (1) full cream milk should be secured for artificially fed infants when possible; failing that, (2) full cream dried milk or (3) full cream unsweetened condensed milk. (2) is preferred to (3), and, in case of ignorant or careless mothers, even to (1), in order to prevent spread of infection

and intestinal disorders. In all cases where (2) or (3) are used, an extra anti-scorbutic should be given (see below).

Sweetened condensed milk is undesirable for the reason that the degree of dilution required by the high sugar content renders the food, as prepared, deficient in the fat-soluble (anti-rachitic) factor as well as in fat and protein.

Milk and butter are the best sources of the anti-rachitic (or fat-soluble) factor for young and growing children; margarines made from animal fats are also valuable; those made from vegetable oils are to be condemned. If there is a shortage of butter it should be reserved for children, but if totally lacking the deficiency can be replaced by cod-liver oil and other fish oils, or by eggs. If all animal fats are unavailable, pea-nut oil should be selected in preference to other vegetable oils for preparation of margarines, &c., and some effort should be made to utilize the fat-soluble vitamine contained in green leaves.

Green leaves are a cheap and readily available source of the fat soluble vitamine, and adults can probably maintain good health when animal fats are substituted by vegetable fats if green-leaf vegetables are consumed in fair quantity. In case of this vitamine, the loss involved in ordinary cooking is not serious. Unfortunately infants or very young children cannot take green vegetables in the ordinary way, but the juices expressed from cabbages and other green-leaf vegetables, raw or even after steaming (not immersing in boiling water) for a few minutes, might be given even to infants if all other sources of this most necessary vitamine have failed.

Purées, carefully prepared from cooked spinach or lettuce, can be tolerated in small quantities (one teaspoonful daily) by many young infants, and the amount taken can be increased regularly with age.

In cases where rickets or growth failure or xerophthalmia are already well established, a daily dose of cod-liver oil is essential in addition to all other procedure.

Pregnant and nursing mothers should have as liberal a supply of the fat-soluble factor as is possible. Rickets is not confined to artificially fed children. Breast-fed children depend for an adequate supply of this factor on the milk, which in turn depends upon the diet of the mother.

(3) Prevention of Scurvy.

Use of germinated seeds. If fresh vegetables or fruit are scarce or absent an anti-scorbutic food can be prepared by moistening any available seeds (wheat, barley, rye, peas, beans, lentils) and allowing them to germinate. It is necessary, of course, that these should be in the natural whole condition, not milled or split. The seeds should be soaked in water for 24 hours, and kept moist with access of air for 1–3 days, by which time they will have sprouted. This sprouted material possesses an anti-scorbutic value equal to that of many fresh vegetables, and should be cooked in the ordinary way for as short a time as possible.

In case of shortage it should be remembered that salads are of more value than cooked vegetables. The extent to which the anti-scorbutic factor is destroyed during cooking depends chiefly upon

the time employed. When supplies are limited vegetables should be cooked separately and for as short a time as possible ; they should not be cooked for long periods with meat in soups or stews.

Preserved foods, with a few exceptions, may be regarded as devoid of the anti-scorbutic principle. Lemon juice retains some value in this respect ; canned tomatoes (and presumably other tinned acid fruits) have also anti-scorbutic value. *Canned vegetables are useless for prevention of scurvy, as also are dried vegetables.*

Infantile scurvy must be considered separately as many of the above foodstuffs are unsuited to infants or young children. To avert danger all artificially nourished infants should receive an extra anti-scorbutic. Cow's milk, even when raw, is not rich in the anti-scorbutic vitamine ; when heated, dried, or preserved, the amount contained is still further reduced. The most suitable anti-scorbutic material to use is fresh orange juice, 1–3 or 4 teaspoonfuls (5–15 c.c.) daily, according to age. Raw swede (or, if unavailable, turnip) juice is a potent anti-scorbutic, and an excellent substitute for orange juice ; to obtain the juice the clean cut surface is grated on an ordinary kitchen grater and the pulp obtained is squeezed in muslin. Tomato juice, even from canned tomatoes, and grape juice can also be used ; the latter is, however, less potent than orange juice, and a larger dose should be given.

Pregnant and nursing mothers. If babies are breast fed it is important that the pregnant and nursing mother should receive an adequate supply of anti-scorbutic food in her diet. The popular belief that green vegetables are harmful in such cases is often without foundation. Infantile scurvy is not unknown in breast-fed children.

It is evident that many of the above deficiency diseases are rife among the populations of Central and Eastern Europe. It is essential, therefore, that the principles set forth in the preceding paragraphs should be fully understood by all persons engaged in administering relief to these districts.

Signed on behalf of the Committee,

F. G. HOPKINS, *Chairman.*

HARRIETTE CHICK, *Secretary.*

June, 1919.

(*Correspondence should be addressed to*

The Secretary,

The Committee on Accessory Food Factors,

The Lister Institute,

Chelsea, London, S.W. 1.)

The Distribution of the Three Accessory Factors in the Commoner Foodstuffs.

Classes of foodstuff.	*Fat-soluble A or anti-rachitic factor.*	*Water-soluble B or anti-neuritic (anti-beri-beri) factor.*	*Anti-scorbutic factor.*
Fats and oils.			
Butter	+ + +	0	
Cream	+ +	0	
Cod-liver oil	+ + +	0	
Mutton fat	+ +		
Beef fat or suet	+ +		
Pea-nut or arachis oil	+		
Lard	0		
Olive oil	0		
Cotton-seed oil	0		
Coco-nut oil	0		
Coco butter	0		
Linseed oil	0		
Fish oil, whale oil, herring oil, &c.	+ +		
Hardened fats, animal or veg. origin	0		
Margarine prepared from animal fat	Value in proportion to amount of animal fat contained		
Margarine from vegetable fats or lard	0		
Nut butters	+		
Meat, fish, &c.			
Lean meat (beef, mutton, &c.)	+	+	+
Liver	+ +	+ +	+
Kidneys	+ +	+	
Heart	+ +	+	
Brain	+	+ +	
Sweetbreads	+	+ +	
Fish, white	0	very slight, if any	
„ fat (salmon, herring, &c.)	+ +	„	
„ roe	+	+ +	
Tinned meats	?	very slight	0
Milk, cheese, &c.			
Milk, cow's whole, raw	+ +	+	+
„ skim „	0	+	+
„ dried whole	less than + +	+	less than +
„ boiled „	Undetermined	+	„
„ Condensed, sweetened	+	+	„
Cheese, whole milk	+		
„ skim	0		
Eggs.			
Fresh	+ +	+ + +	? 0
Dried	+ +	+ + +	? 0
Cereals, pulses, &c.			
Wheat, maize, rice, whole grain	+	+	0
„ germ	+ +	+ + +	0
„ „ bran	0	++	0
White wheaten flour, pure cornflour, polished rice, &c.	0	0	0
Custard powders, egg substitutes, prepared from cereal products	0	0	0
Linseed, millet	+ +	+ +	0
Dried peas, lentils, &c.		+ +	0
Peaflour (kilned)		0	0
Soy beans, haricot beans	+	+ +	0
Germinated pulses or cereals	+	+ +	+ +

Classes of foodstuff.	*Fat-soluble A or Anti-rachitic factor.*	*Water-soluble B or anti-neuritic (anti-beri-beri) factor.*	*Anti-scorbutic factor.*
Vegetables and fruits.			
Cabbage, fresh	+ +	+	+ + +
„ „ cooked		+	+
„ dried	+	+	very slight
„ canned			„
Swede, raw expressed juice			+ + +
Lettuce	+ +	+	
Spinach (dried)	+ +	+	
Carrots, fresh raw	+	+	+
„ dried	very slight		
Beetroot, raw, expressed juice			less than +
Potatoes, raw	+	+	
„ cooked			+
Beans, fresh, scarlet runners, raw			+ +
Onions, cooked			+ (at least)
Lemon juice, fresh			+ + +
„ preserved			+ +
Lime juice, fresh			+ +
„ preserved			very slight
Orange juice, fresh			+ + +
Raspberries			+ +
Apples			+
Bananas	+	+	very slight
Tomatoes (canned)			+ +
Nuts	+	+ +	
Miscellaneous.			
Yeast, dried		+ + +	
„ extract and autolysed	?	+ + +	0
Meat extract	0	0	0
Malt extract		+ in some specimens	
Beer		0	0

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